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MASTER OF SCIENCE

A Proof-of-Concept Study to evaluate the benefit from add-on therapy with montelukast versus salmeterol in children with asthma carrying the Arg/Arg-16 β2-receptor genotype

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A PROOF-OF-CONCEPT STUDY TO EVALUATE THE BENEFIT FROM ADD-ON THERAPY WITH MONTELUKAST VERSUS SALMETEROL IN CHILDREN WITH ASTHMA CARRYING THE ARG/ARG-16 β_2-RECEPTOR GENOTYPE

MSC BY RESEARCH

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DECLARATION

Author's Declaration

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OUTLINE OF THESIS

This thesis details my journey as a research nurse describing the design, pre-study application process and performance of one of the first genotype-stratified randomised controlled studies in children with asthma.

Background

β_2-agonist response may be affected by β_2 adrenoreceptor genotype (*$ADRB_2$*). The *arginine-16* (*Arg 16*) variant of this gene predisposes to exacerbations in asthmatic children and young adults, particularly in those exposed to regular salmeterol. There follows the presentation of a proof-of-principle randomised controlled trial of add-on therapy to inhaled steroids, with either oral montelukast or inhaled salmeterol, on children carrying the susceptible *Arg-16* genotype over a 1-year period.

Methods

I have explored methodological issues of importance in the design of this complex study in children. I have also analysed some of the practical aspects of the ethics-related issues associated with the study, the response of parents and children, and how these issues were managed within the context of this study.

Results

Asthmatic children with the *Arg-16* genotype appear to have better asthma control when prescribed montelukast compared to salmeterol, when added to inhaled corticosteroid, over a 12 month period. Sixty two asthmatic children with the *Arg-16* genotype were randomised to receive montelukast 5/10mg once daily or salmeterol twice daily as add on therapy to inhaled fluticasone for 1 year. School absences (the primary outcome) were reduced with montelukast compared to salmeterol: $p = 0.005$. Salbutamol use was also reduced with montelukast compared with salmeterol: $p < 0.0001$, and improvements were also found in symptom and quality of life scores with montelukast in comparison to salmeterol.

Conclusions

Montelukast may be suitable as tailored second line controller therapy instead of salmeterol in asthmatic children expressing the susceptible *Arg-16* genotype - moving towards a personalised medicine approach to management. The study has suggested the need for larger studies which explore the role of genotyping in improving the care of children with asthma. It has also provided the opportunity to explore the issues around consenting and recruiting children within the context of prospective genotyping progressing to randomisation, and the subsequent allocation of children to specific pharmacological interventions on the basis of genotype.

SECTION 1 - INTRODUCTION

Chapter 1

ASTHMA IN CHILDREN

Incidence in children

The incidence of asthma in both adults and children in the UK is one of the highest worldwide.[1] An estimated 1.1 million children are said to have asthma in the UK[2], making asthma the most common chronic disease of childhood and one of the main reasons for childhood hospital admissions.[3] Work in Scotland has estimated that 1 in 11 children are receiving treatment for asthma.[4]

The onset of asthma commonly occurs during preschool years.[5] A high percentage of pre-school children will have episodes of wheeze, cough and increased work of breathing associated with viral upper respiratory infection. Studies have found that up to half of those children who had symptoms in the first year of their life will no longer experience such episodes by early school years.[6] The patterns of expression of childhood asthma that persist into adult life have been explored and distinct asthma phenotypes (transient wheezing, non-atopic wheezing, and atopy-associated asthma) have been identified.[7] Several factors, such as age at presentation, gender, severity of previous wheezing episodes, co-existing atopic disease and family history of atopy, are associated with the development of asthma in childhood.[8]

Genetic & Environmental predisposing factors for Asthma

The exact cause of asthma is not completely understood but it is thought to result from a combination of genetic make up and the environment to which the individual is exposed.[9,10] Environmental factors include indoor allergens (e.g. house dust mites, pollution or pet dander) or outdoor allergens such as pollens, moulds, tobacco smoke and chemical irritants. An increasing number of genes that may be predictive of asthma have also been studied.[11]

How does asthma affect children?

Having asthma can restrict the ability of children to participate in everyday activities, cause school absences[3] and affect performance at school because of night disturbances.[12] In quality-of-life studies, children with asthma rated being well enough to attend school and participate in sports as important to them.[12] Uncontrolled asthma and the possibility of an asthma attack can influence family decisions about activities such as holidays and can affect

social well-being in childhood.[13] Studies have shown school attendance of children with asthma can be poorer, and the ability to take part in physical activities is affected in comparison to healthy non-asthmatic children.[14] More school absence is reported due to asthma than any other chronic disease. In the US up to 60% of children with asthma miss school annually due to respiratory symptoms.[15,16] Children with obesity are at increased risk of developing asthma. The reasons for this have not yet been defined but diet, reduced physical activity or genetic alterations that increase tendency towards both obesity and asthma are all said to contribute.[17]

Pharmacological Treatments for Children's Asthma

There are two main types of asthma pharmacological treatments (1) reliever drugs that target acute airway bronchoconstriction and (2) preventer drugs used to reduce airway inflammation and the severity and frequency of exacerbations. The main reliever medications, and the most commonly used asthma treatments, are β_2 agonists e.g. salbutamol. For moderate and severe persistent asthma reliever treatment is combined with inhaled corticosteroids – preventer drugs - to reduce the severity of airway inflammation.[18]

National Guidelines

The British Thoracic Society Asthma Guidelines classify treatment recommendations into five steps depending on the severity of the child's asthma.[18] Step I of asthma treatment is reliever inhaler used only as needed and step II is daily inhaled corticosteroids with reliever as needed. At step III, long-acting β_2-agonists are added when symptoms are inadequately controlled by inhaled steroids alone.[18] If a combination of inhaled long-acting β_2-agonist and inhaled steroids does not control asthma symptoms adequately, montelukast, a leukotriene receptor antagonist, can be added as additional therapy. Asthma that is uncontrolled at step III is managed through the addition of further levels of medication such as oral theophylline

(step IV). Steps IV and V of the guidelines advise increased levels of inhaled corticosteroid and finally the addition of daily oral corticosteroids.[18]

Variations between Childhood and Adult Asthma

A diagnosis of asthma can be assumed when a child presents a pattern of such respiratory symptoms where no alternative causative explanation can be provided.[18] There are several issues unique to children that must be kept in mind when asthma characteristics are studied in paediatric age-groups. There are important differences in the clinical features and pathological characteristics of asthma between children and adults.[19,20] Different wheezing phenotypes exist in children that potentially affect children to varying degrees at different age groups.[5] In addition, the adverse effects of asthma treatments may also vary between children and adults.[21,22] Side effects that may not affect adults may be very important in children e.g. the effect of high dose inhaled corticosteroids on growth in children.[18]

Chapter 2

THE PATHOLOGY OF CHILDREN'S ASTHMA

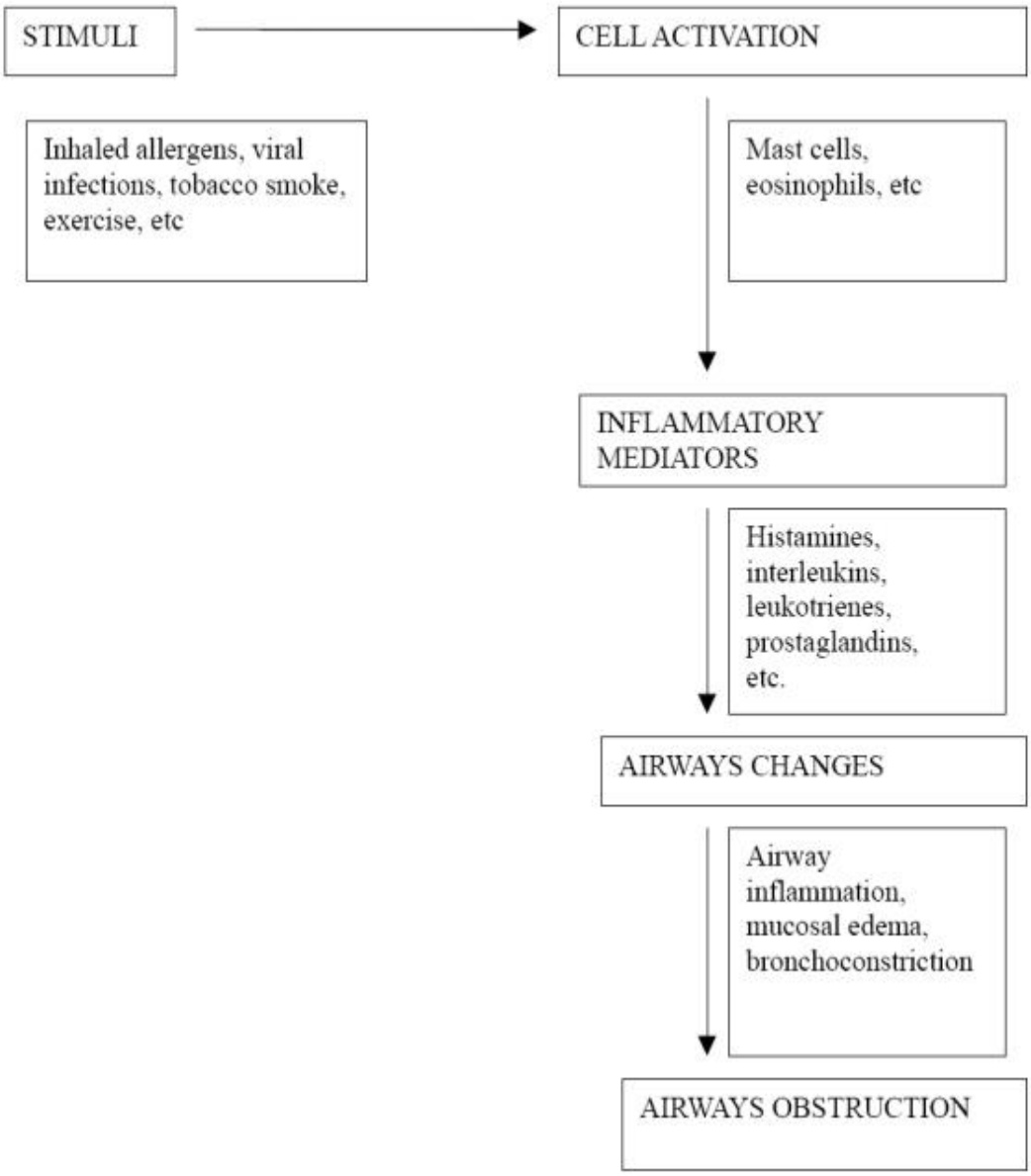
This chapter briefly outlines the pathology of children's asthma and I have aimed to relate this to the pharmacological effects of the main two asthma drugs used in the thesis study – salmeterol and montelukast.

Pathology of Asthma Exacerbations

The clinical symptoms of asthma - wheeze, cough, chest tightness and breathlessness occur as a result of chronic inflammation of the airways and acute inflammatory episodes (exacerbations).[10] The inflammatory responses in an exacerbation, involve the action of cells such as mast cells, lymphocytes, eosinophils, neutrophils, macrophages and also chemical mediators called leukotrienes, which are produced by these cells.

Inflammation of the airways and airway reactivity cause bronchospasm, mucus secretion and oedema of bronchial tissue.[20] Airway wall epithelial cells mix with mucus to form thick plugs which can block the narrow diameter of a child's airway and restrict air flow through the bronchioles.

Pathogenesis of airway obstruction in asthma



Reproduced by kind permission Harmanci K (2007)
'Montelukast: its role in the treatment of childhood asthma'
Therapeutics and Clinical Risk Management Vol 3 Issue 5 October pp 885-892.

Pathology of inhaled β_2 agonists in Asthma

β_2 agonists are one of the first choice medications to treat exacerbations. Following inhalation, asthma drugs are deposited on the mucosal fluid lining of the airways. The target of β_2 agonists are β_2-adrenoceptors (β_2ARs) in the bronchial smooth muscle. β_2ARs are protein receptors on the cell surfaces that signal to the interior of the cell through multiple mechanisms causing the cellular response to inhaled β_2 agonist.

Signaling cascade involved in the β_2 receptor activation

By binding to the receptor, β_2 agonists such as salbutamol initiate intracellular biochemical events. The process of β_2 receptor activation is mediated by adenylyl cyclase stimulation and subsequent cyclic adenosine monophosphate (cAMP) formation. cAMP messenger activates protein kinase (PKA), which transfers the effects of salbutamol into the cells, resulting in bronchodilation by relaxing the smooth muscles of the airways from the trachea to the terminal bronchioles.[21]

Arg/Arg 16 Single Nucleotide Polymorphism of the β_2 adrenergic receptor

A number of Single Nucleotide Polymorphisms (SNP's) in the β_2 adrenergic receptor gene have been studied. It is believed that the polymorphic changes at position 16 have normal agonist binding at the receptor, but there is less interaction between the β_2 receptor and β_2 agonist drugs in individuals who have this particular genetic variation.

Down regulation of the β_2 adrenergic receptor

Down regulation occurs during prolonged agonist activation and is a decrease in the number of cell surface receptors. The mechanisms leading to the process are highly cell dependent. The Arg/Arg16 polymorphism confers relative protection against down-regulation by endogenous catecholamines, making individuals susceptible to uncoupling and internalisaton

of receptors known as desensitisation followed by a decrease in receptor density and receptor gene expression known as down regulation.[23]

Short acting and Long acting β_2 agonists

There are two main types of β_2 agonists used in children's asthma – short acting β_2 agonists and long acting β_2 agonists (LABAs) e.g. salmeterol. The degree of bronchodilator response of β_2 agonists is related to the concentration of drug in the smooth muscle cells, and the extent of activation of the receptor by the β_2-agonist.[23]

The exact mechanisms by which salmeterol causes prolonged bronchodilation is not fully understood but it is thought that the drug can be stored in cell membranes in the airways and therefore accessible to the β_2ARs for a longer period.[24] In comparison the short acting β_2-agonist will diffuse more readily through the airway tissue and is dispersed quicker.

Pathology of Montelukast in Children's Asthma

Leukotrienes are key mediators of the inflammatory airway responses. They have both bronchoconstrictor and inflammatory roles in the pathophysiology of chronic asthma.[25] Montelukast inhibits the leukotriene pathway and acts through a different pathway to that involved when the β_2AR is activated. Montelukast is known to be effective in children's asthma.[25]

Chapter 3

MEASURES OF ASTHMA CONTROL

In this chapter measures of asthma control used in the study are discussed as outcome measures, showing examples of evidence to support their effectiveness in clinical trials and contraindications for their use in both clinical practice and research involving children.

There are issues unique to children that clinicians and researchers should be mindful of when assessing symptoms and evaluating treatment outcomes. Children may not recognise symptoms or have difficulty describing symptoms. Parents can report symptoms but there may be differences between reports from children and their parents.

In asthma clinical trials, objective measures such as lung function, airway inflammation and need for short-acting inhaled β_2 agonist have traditionally been used to evaluate the effect of asthma interventions. Child and parent-reported outcome measures describing quality-of-life can also be used to provide both quantitative and qualitative data. Outcomes commonly used in routine clinical practice, such as symptoms scores, frequency of periods of increased symptoms and peak expiratory flow rate variability are also useful as clinically significant end-points for evaluating the role of asthma interventions. A brief discussion of commonly used outcome measure follows, with particular emphasis on the outcome measures used in the main thesis study.

School Absence as a result of Asthma

Absence from nursery or school due to asthma is a relevant outcome measure to evaluate asthma control because being able to attend school matters to children and parents. In

studies, it provides a qualitative assessment of response to asthma treatment which can be measured retrospectively either from parent/child recall or school records.[3] In clinical practice a record of time off school is a useful indicator of control. Comparisons of school absence can be made among study groups where participants have been randomised, or where a comparable participant group is included.[26] A study carried out in Tayside used school absence as an outcome indicator and showed that children prescribed inhaled salmeterol had more school absence in comparison to other asthma treatments.[27] It could be argued that parent/child recall may be inaccurate but in a study where the child is closely monitored with a number of visits over a short period the likelihood of inaccuracy will be less. School absence data can be obtained from school records but this may be of limited value if the exact cause of absence is not recorded.

Exacerbations

Exacerbations of asthma are defined as an acute worsening of chronic asthma requiring one or more of the following (1) systemic use of corticosteroids, (2) asthma-specific emergency department attendance or hospital admissions[28] or (3) increased use of short acting β_2 agonists as rescue inhaler.[29] As a measure of control, exacerbations indicate changes in clinical status. However visits to emergency departments and hospital admissions are usually infrequent in children. In contrast courses of prednisolone[18] and β_2 agonist use can be recorded as more frequent events over a shorter period of time.

Mild clinical exacerbations in children can include increased nocturnal symptoms, breakthrough symptoms, and asthma-related school absences.[10] These markers are observed more frequently over a shorter period and smaller numbers needed to observe an effect. Some studies have combined all these levels of exacerbations into asthma control days. [30]

Summary of Clinical Features

In clinical trials a summary of asthma symptoms from the child and/or parent are commonly used to give an index of response to treatment.[31,32] During an asthma exacerbation, children will experience shortness of breath, cough and/or wheezing. Between attacks, they may be asymptomatic or describe mild-to-moderate symptoms that could be related to exertion, or nocturnal awakening due to narrowing of the airways at night. These clinical features that change over time altering asthma status are useful to study the course of the disease. Asthma symptoms in children tend to be more variable in comparison to adults.[33]

Use of inhaled short-acting β_2 agonists

Short acting β_2 agonist use has been widely used as an indicator asthma morbidity and a marker of asthma control in children.[34,35] This can be obtained from parent/child recall which would rely on their ability to recognise symptoms and record frequency of use. Information on frequency of prescription collection could also be obtained via Primary Care.

Pulmonary Function

Measuring pulmonary function by means of spirometry is one of the more traditional asthma assessment methods in clinical settings. It is easily obtainable in older children and can provide an objective measure of airway inflammation and pathological changes in the airway over time. Spirometry measurements, such as the volume of air that can be forcibly expired during the first second of expiration after a maximal inspiration (FEV1), and peak expiratory flow rate (PEFR), the maximal flow rate achieved during forced exhalation, can effectively demonstrate the action of bronchodilator treatment.

However, it can be more difficult to quantify the effect of a treatment using pulmonary function alone in children because the majority have lung function measurements within the

normal range limiting the potential to show benefit.[26,27] Studies of asthmatic children with poorly controlled disease had no effect on pulmonary function following the addition of long acting β_2 agonists.[36, 37]

It can also be argued that pulmonary function changes may not associate with outcomes that are relevant to children and their families. Changes in day to day symptoms may not be fully captured with pulmonary fucntion alone. Children and their families may perceive some asthma symptoms as more troublesome than others and may report benefits from asthma treatment which cannot be explained on the basis of clinical pulmonary function.

Quality-of-life related outcomes

In contrast to physiological outcome measures used to assess asthma, the aim of quality of life measurement is to assess the impact asthma has on the child's daily life and emotional well-being. Increasingly, the National Institute for Health and Clinical Excellence (NICE) and other bodies are placing more emphasis on qualitative assessments such as the Paediatric Asthma Quality of Life Questionnaire, as outcome measures, to assess response to asthma treatment in children.[38,39]

Quality of life tools for children with asthma measure emotions, asthma severity/symptoms, missed school days, activity limitations and visits to the emergency department[40] and are used to capture the effects of treatments and asthma control as perceived by the children themselves.

Clinical and laboratory measurements of asthma do not always correlate with each other, and information from patients is valuable in evaluating the asthma status. Studies in adults with asthma have shown only small links between clinical outcomes and how patients feel and

function daily.[41 42] If only traditional asthma clinical end-points such pulmonary function are measured in clinical trials, it is possible that important patient perceived benefit to treatments may be missed.[43,44] Thus to gain a full picture of the impact of asthma on the lives of sufferers, measurements of health-related quality of life should be considered.

Asthma diaries

Measuring peak flow can provide a quantitative measure of asthma, to help detect early changes that may require treatment or to evaluate responses to changes in therapy. Current asthma guidelines recommend peak flow monitoring during exacerbations of asthma to help determine the severity of exacerbations. Change from baseline in morning peak flow as recorded in daily diary cards can be used in research studies.[45] It is important to acknowledge that there is a risk that children will not maintain adherence and the potential for incorrect readings should be recognised as a limitation to long-term peak flow monitoring.[45]

Inflammatory Biomarkers

Biomarkers in asthma can be defined as measurements in the laboratory associated with the biology or physiology of the clinical disease process in asthma.[46] Inflammation plays a major role in the pathophysiology of asthma and mediators of airway inflammation in exhaled breath, urine, sputum, blood or saliva are used to monitor asthma and to evaluate response to interventions.[47]

Exhaled Nitric Oxide (FeNO)

Exhaled breath nitric oxide (FeNO) may reflect eosinophilic airway inflammation in asthma. There is a significant relationship between FeNO measurements and eosinophilic airway inflammation. In children, management based on FeNO as an end point has been associated with improvements in airway hyper-responsiveness, but not with clinical benefits as reflected

by patient-experienced outcomes, such as symptoms or the need for rescue medication.[30] In addition, most studies have not shown a reduction in exhaled FeNO levels at step III asthma treatment with inhaled long-acting beta agonists in asthma.[48,49]

Eosinophilic cationic protein (ECP)

The status of eosinophils is known to be an indirect marker of airway inflammation in asthma. Total eosinophil count reflects asthmatic activity and has been shown to be useful for regulating steroid dosage and for early detection of exacerbations.[50] A number of studies have indicated that the assessment of eosinophil-derived proteins in serum or urine samples may be useful for monitoring disease activity in asthma. The eosinophil granule proteins often function well as inflammatory markers when used in controlled clinical studies and are therefore useful research tools.

Eosinophilic cationic protein (ECP) has been most frequently used as a marker of inflammation. It is produced and secreted by eosinophils.[50] ECP is used in clinical trials for monitoring disease activity. However obtaining levels of serum ECP either from blood, or sputum samples is invasive for children and may be a deterrent for children to participate in a study.

Based on this information, the research team debated whether the analysis of ECP in saliva may be adequate to enable clinically relevant assumptions on the status of the airways in patients with asthma. However, on overall analysis, it was felt that the current state of understanding of the relationship between salivary ECP levels and asthma symptom control is unclear.

Although there is increasing evidence to support a role for ECP and FeNO as biomarkers of asthma status, in this study it was felt that clinical outcome measures, such as absence from school, and asthma-related quality-of-life, were more likely to indicate benefit for patients, in comparison to ECP or FENO measurements. Thus, on balance, I conclude that a more relevant endpoint for comparison of disease activity in children's asthma may be represented by outcomes such as school absences, other measures of asthma exacerbations, and asthma symptoms scores measured over a certain period of observation during the comparison of interventions. Inflammatory biomarkers can reveal important information about the disease process, but will not capture how the patient feels and is affected by asthma on a daily basis

Chapter 4

ROLE OF GENETIC VARIATION ON ASTHMA CONTROL AND SEVERITY

Polymorphic variations in a number of inflammation-related genes have recently been shown to exert important effects on susceptibility to allergy and asthma. Such variation can also affect asthma clinical phenotype in childhood, thus influencing asthma severity. There has been major progress in this area of research over the past decade, including work that specifically relates to children. The body of literature is very large. However, the concept of genetic regulation of aspects of asthma control, through interaction with environmental factors such as exposure to medication, is of central importance to my thesis. Hence, this section, will review some specific, important examples from recent literature of the effect of genetic variation on asthma severity.

Filaggrin gene variation and the effect of this variation on asthma control in childhood.

Filaggrin (FLG) gene variation

Two independent mutations in the gene (*R501X* and *2282del4*) of the filaggrin gene (*FLG*) have been shown to strongly predispose to childhood eczema and other atopic conditions.[51] The filaggrin gene mutations are common in several white European populations, and in the UK, about 10% of children appear to have a skin barrier resulting from filaggrin gene mutations.

Filaggrin

Filaggrin is a structural protein found within the stratum granulosum cell layer of the epidermis, which maintains the skin barrier function. Filaggrin helps to form a keratin cytoskeleton to compact cells, known as squame. Squame is a cell barrier in the skin,

impermeable to chemicals, that retains water and prevents entry of antigens, allergens and irritants from the environment. Absent or reduced filaggrin in children with the *FLG* gene mutations *R501X* and *2282del* have been found to disrupt barrier formation and possibly permit transcutaneous antigen/allergen/ irritant transfer through the skin.[52]

Effect of Filaggrin variation on asthma control in childhood

The severity of disease in children with established asthma has been shown to be increased in children with the 'at risk' form of the *FLG* gene variation.[53,54] The presence influences controller and reliever medication needs in asthmatic children[53] and leads to a greater risk of asthma attacks.[52] Furthermore, other markers of asthma severity in childhood, e.g. school absences, use of oral steroids, and frequency of hospital admissions appear to be influenced by variations in the *FLG* gene and the effect of these variations on skin barrier function.[54] This influence could be the result of differences in skin barrier function which may result in different patterns of allergen entry through the skin.

Glutathione-S-transferase (GST) variation influences lung function in children with asthma

Glutathione-S-transferase (GST) gene variation

The most studied variants of the *Glutatione-S-transferase* gene are *GSTM1* and *GSTT1*, present in 50% and 20% within white populations, respectively, and also a variant of *GSTP1*, which constitutes 10% of the white population.[55]

Glutathione-S-transferase

Glutathione *S*-transferases are an important family of enzymes involved in the detoxification processes occurring within the human body. Polymorphic variation of the gene's synthesising proteins, can lead to differences in the activity of these enzymes.[57] *GSTM1* and *GSTP1* are

enzymes that protect the airways from oxidative stress, which is linked to asthma pathogenesis. A reduction in the activity of GSTs may lead to an impairment of the detoxification process in the lung and other parts of the body which could contribute to worse asthma. These enzymes that contributing to local detoxification in alveoli and bronchioles also have an important role in the defence mechanism against tobacco smoke.

Glutathione-S-transferase gene variation in children with asthma

A number of studies have investigated the relationships between different polymorphisms of *GST* and aspects of asthma clinical phenotype in children. Genes encoding the *GSTs* have been implicated in various aspects of immune responses in the pulmonary and cardiovascular systems and there are reports of a modulating effect of genetic variation in *GSTs* on asthma susceptibility.[55] and that *GSTM1* is associated with asthma risk in children exposed to tobacco smoke

Smoke exposure has been linked to poorer asthma outcomes. In *GSTM1*-null children of school age, *in utero* exposure to smoking is associated with an increased prevalence of early onset asthma, asthma with current symptoms, persistent asthma, lifetime history of wheezing, wheezing with exercise, wheezing requiring medication, and emergency department visits in the past year.[56] Variation in the *GSTM1* locus influences lung function in children. This effect was particularly important among children whose mothers smoked during pregnancy.[57] In cross sectional analysis of childhood asthma, it was observed that in utero exposure to maternal smoking was associated with increased risk of asthma/wheeze only among carriers of the *GSTM1* null genotype.[58] Tobacco smoke exposure was also found to exert a particular adverse effect on peak expiratory flow rate in teenagers with asthma carrying the common *GSTM1* null variation.[59]

Peroxisome proliferator activated receptor – gamma (PPAR-gamma) variation and asthma

Peroxisome proliferator activated receptor – gamma variations

Variation in the gene synthesising the peroxisome proliferator activated receptor – gamma (*PPAR gamma)* protein represents another example of the effect of gene change on asthma severity in childhood. A number of functionally important polymorphisms of the PPAR gamma gene, *Pro12Ala, C1431T,* and *C-681G,* are present in the population in Scotland.

Peroxisome proliferator activated receptor – gamma

PPAR gamma is a nuclear receptor, and has an anti-inflammatory role in the human body. The role of this protein has been particularly investigated within the context of vascular disease [60] and cancer[62] *PPAR gamma* is expressed in the lung and is likely to have similar, although currently unexplored, roles in the lung.

Peroxisome proliferator activated receptor – gamma associated with increased risk asthma exacerbations

Work has shown that *ProC* represents an 'at-risk' polymorphism for asthma.[61] This variant is commonly present and is associated with increased risk of asthma exacerbations in children. Specifically, the *ProC* genotype was associated with increased school absences and hospital admissions over a period of 6 months. This paper concludes that common genetic variation at the *PPARG* locus may play an important role in modulating the long term control of asthma in children and young adults.[62]

Beta$_2$ adrenoreceptor gene polymorphism may result in variability in response to asthma treatments

β_2 adrenoreceptor gene polymorphisms (*ADRB$_2$*)

In the human population, a number of polymorphisms of the β_2 adrenergic receptor gene have been found, at least 3 of which result in receptors that have different properties. Two common variants at amino acid positions 16 (Gly16Arg) and 27 (Gln27Glu) have been the focus of clinical studies in asthma. Specifically, the polymorphism *Arg/Arg-16* of the β_2 adrenergic receptor has been linked with increased symptoms and exacerbations in children with asthma.[27,63]

Prevalence of Arg/Arg-16 gene variation

About 15% of children of Northern European origin are homozygous for the *Arg/Arg-16* gene variation. Approximately 45% of the population carries one arginine and one glycine residue at position 16. Thus about 15 % of the population may be susceptible to the effects of differential β_2 agonist response, although it is possible that a much larger population may be partially affected.[27,64]

Beta$_2$adrenoreceptor

Variability in response to asthma treatments can be influenced by variation in the structure of the receptor molecules that bind to asthma medicines. An important receptor in this context is the adrenergic β_2 receptor that binds to two of the most common medicines used in children's asthma, inhaled β_2-agonists - salbutamol and salmeterol. Inhaled β_2-agonists cause dilation of constricted airways by binding to the β_2-adrenoreceptors on airway smooth muscle. Naturally occurring polymorphisms in the β_2 adrenoreceptor gene (*ADRB$_2$*) might alter the function and expression of the adrenoreceptor and therefore affect response to short and long acting *β_2*-agonists.[64] The β_2-adrenergic receptor has been found to have significant genetic variability in its structure resulting in differences in the way the receptor functions.[64]

Beta$_2$ adrenoreceptor gene variability in response to asthma treatments

The hypothesis that this variation may lead to differences in treatment response in children's asthma has been tested.[27,63] Past studies have associated the *Arg/Arg-16* gene variation, with a reduced response to short-acting β_2-agonists[65], as well as perhaps to long-acting beta$_2$-agonists.[66] This has led to concerns that the use of combined inhaled corticosteroid (ICS) and long-acting β_2 agonist medications, would result in poor clinical outcomes in patients with polymorphisms of this gene. Observational studies in Scottish children with asthma have consistently shown a role for β_2 adrenoreceptor variation in β_2 agonist response.[27,63] Hence, there is a need to study the role of this receptor on therapeutic response in Scottish children with asthma

Conclusion

I have reviewed a number of studies that have identified individual candidate gene variants that are associated with a more severe asthma phenotype. However, in most clinical situations, such variants have limited use in the prediction of disease severity, since most of them confer a relatively small risk. In other disease areas, such as multiple sclerosis, prostate cancer and type II diabetes, combinations of individual gene variants have a cumulative effect, conferring larger associations with susceptibility to these diseases.[67] In addition to an increase in susceptibility, combinations of individual variants may also be expected to determine the severity of chronic disease phenotype in patients with already existing disease. In childrens asthma, for example, a greater cumulative risk of severe asthma associated with the presence of multiple 'risk' variants, that are known to be individually associated with increased asthma severity, may determine why one child progresses towards regular multi-drug therapy and relatively frequent requirements of courses of oral steroids, while another remains well-controlled on occasional puffs of 'reliever' medicine or a modest, regular dose of inhaled steroids.

Some forms of genetic variation may influence the response to medication. This is particularly true of the β_2 adrenoreceptor gene variation, as discussed above. The clinical importance of the variation could be tested by randomly allocating patients carrying the “at-risk” genotype to two different forms of medication, one of which is ‘standard’ and the other is the alternative medication, to which they are more likely to respond, if the hypothesis of reduced efficacy based on gene status is correct.

Chapter 5

CLINICAL TRIALS IN ASTHMA FOCUSING ON THE RELATIONSHIP BETWEEN *BETA$_2$* AGONIST USE AND *ARG/GLY* VARIATION

I have performed a literature review using a pre-defined search strategy in order to identify, and subsequently study, clinical trials in asthma that have used stratification by genotype.
The search was performed using the Pubmed and Web of Knowledge databases. The keywords 'asthma', 'genotype' and 'trial' were used for the search. I could not identify any randomised controlled trials with stratification by genotype in the field of children's asthma. I explored studies of participants with asthma carrying one or two copies of *Arg16* genotype, where genotype was established retrospectively or prospectively. I concentrated on current studies, restricting my searches to the period 2000 – 2014, in order to develop a fuller understanding of the current literature.

Studies involving short-acting ßeta$_2$-agonists (SABAs)

One of the first genotype-stratified, prospective placebo-controlled trials in adults in this area was the BARGE trial (Beta-Adrenergic Response by Genotype).[68] This study was designed to compare the effects of regularly scheduled use of inhaled albuterol in patients with mild to moderate asthma with either *Arg/Arg* or *Gly/Gly* genotype variation at the β_2 -adrenoreceptor**.**
The hypothesis for this study was that regular administration of inhaled albuterol, compared to placebo, will have a detrimental effect on asthma control in subjects with the *Arg/Arg 16* genotype. In contrast, regular albuterol will have no significant effect, compared to placebo, in patients with asthma of a similar baseline severity with the *Gly/Gly* genotype.

Seventy eight participants – adults - were randomised to either regular salbutamol or placebo for 16 weeks, followed by a crossover in treatment for another 16 weeks. *Arg/Arg* subjects had lower peak expiratory flow rates during treatment with regular albuterol compared with placebo.

Litonjua *et al* used a novel family screening algorithm based on the statistical power of each single polymorphism (SNP) to rank 844 SNPs in 11 bronchodilator response candidate genes in a cohort of 209 children and their parents participating in the Childhood Asthma Management Program (CAMP).[69]

Martinez (1997) performed a birth cohort study to compare response to a single dose of salbutamol.[70] The participants were of Hispanic or Caucasian descent. Two hundred and sixty nine children were genotyped and asked to report episodes of wheezing during the previous year. Children who were homozygous for *Arg16* were 5.3 times more likely to respond to a single dose of salbutamol than the children who were homozygous for *Gly16*.

Ethnic-specific pharmacogenetic differences in the effect of *Arg16Gly* genotype on drug response were demonstrated in a study of Latino Americans with asthma. Significant associations were found between the number of Arg16 alleles and responsiveness to a single dose of salbutamol among asthmatic Puerto Ricans with a baseline FEV_1<80% predicted. This relationship was not present in Mexican asthmatics.[71]

The relationship between the long-term use of SABAs and the Arg16Gly genotype has also been studied. Participants were stratified by genotype for *Arg16Gly* and *Glu27Gln* and then randomised to receive regular plus as-needed salbutamol or as-needed salbutamol.[69] Regular SABA use was associated with a decline in morning and evening PEFRs in adult patients who were homozygous for Arg16, but not other genotypes.

Studies involving long-acting βeta$_2$-agonists (LABAs)

Bleecker *et al* performed a similar analysis but found contradictory results, with no evidence of association between the *Arg16Gly* genotype and response to salmeterol in combination with inhaled corticosteroid (ICS).[73] This led to two larger, genotype-stratified, randomised controlled trials. In the LARGE (Long-Acting Beta Agonist Response by Genotype) study[73], a total of 87 adult patients were randomised to 18 weeks of treatment with salmeterol or placebo with a subsequent crossover. Open-label use of beclomethasone and ipratropium bromide was allowed throughout the trial. The LARGE study examined polymorphisms of *ADRB$_2$* in participants previously enrolled in clinical trials involving the LABA salmeterol. They found that *Arg/Arg* patients had a decrease in PEFR and diminished therapeutic response to the regular use of salmeterol, compared with those with *Gly/Gly*. Both *Arg/Arg* and *Gly/Gly* patients showed improved PEFRs after treatment with salmeterol. However, this study's impact was limited by its small sample size.

Recently, Bleecker *et al*[74] published the results of a large genotype-stratified clinical trial where 544 adult asthmatic subjects with *Arg/Arg*, *Gly/Gly* or *Arg/Gly* at codon 16 were randomised to salmeterol alone or in combination with inhaled fluticasone propionate, for 16 weeks. Both *Arg/Arg* and *Gly/Gly* subjects showed improvement in morning PEFR with salmeterol alone and salmeterol in combination with fluticasone propionate, although the differences in PEFR were not statistically significant. Furthermore, *Arg16Gly* genotypes did not modify other secondary treatment responses, including evening PEFR, FEV_1, as-needed ipratropium bromide use and percentage of symptom-free days. The authors conclude that there was no evidence of a pharmacogenetic effect of *ADRB$_2$* gene polymorphisms on salmeterol response, in particular at the *Arg16Gly* position.

The SMART trial (Salmeterol Multicenter Asthma Research Trial)[75] is a randomised controlled trial comparing the safety of salmeterol or placebo added to usual asthma care. Interim analysis

showed that significantly more African Americans prescribed salmeterol experienced respiratory-related death or life-threatening experiences compared with those prescribed placebo. Because of these preliminary findings in African Americans and difficulties in enrollment, the trial was stopped prematurely.

Overall, although the combined findings from the LABA studies may be reassuring, the use of LABA monotherapy remains contraindicated in the treatment of asthma because of the increased risk of severe exacerbation of asthma symptoms in some patients. Various studies have identified a role for *Arg/Gly* variation on the β_2 receptor gene that may influence the response to SABAs and LABAs. This pharmacogenetic response also varies by ethnic group. Most of the literature has focused on the use of SABA and LABAs with regard to safety. This is important as these medicines represent a widely prescribed class of asthma medication.

The retrospective observational study carried out in Tayside in 2006 found increased exacerbations in asthmatic children with the Arg /Arg 16 polymorphism, especially those using inhaled salmeterol.[63] Data from 546 asthma sufferers between the ages of 4 and 21 years were examined. The population carrying the *Arg/Arg-16* genotype variant (13%) and prescribed inhaled long-acting β_2 agonists, was found to be less well controlled, with 70% of this population having had significant asthma exacerbations within the six month trial observation period. In comparison, exacerbations occurred in only 33 % individuals with the *Gly/Gly* polymorphism of the *ADRB*$_2$. Also of note, it was found that, in the children receiving salmeterol, the adjusted odds ratio showed a 9 fold (p=0.003) greater risk of school absences due to asthma in the Arg/Arg group in comparison to the Gly16 carriers, with an unadjusted odds ratio of 6 (p=0.009). In the cohort not receiving salmeterol, there was no evidence of any genotype-dependent increases in school absences due to asthma (odds ratio=1). In addition, the *Arg/Arg* children on salmeterol had a significantly increased risk of extended school absence of over 1 week from asthma with an adjusted odds ratio of 6 (p= 0.019).

Conclusion

Information about medication effects on asthma provides opportunities to design studies that will guide improvement in asthma care which could lead to further information in how best to target treatments to those patients who would benefit most i.e. to stratify a population needing treatment into those likely or unlikely to respond to treatment.

This forms the background to one of the first randomised controlled studies on a genotype stratified population of asthmatic children. This represents the main study within my thesis.

SECTION 2 - METHODS

Chapter 6

REGULATORY AND LEGAL PROCEDURES AND APPROVALS

In this chapter I would like to describe the processes in which I was involved prior to beginning the clinical phase of the study. The following are mandatory procedures to safeguard the rights and well being of potential participants and assist researchers produce credible results.

Regulatory & Legal Procedures

Regulatory and legal procedures were approved by University of Dundee, Tayside Research Ethics Committee and the Research and Development Department for NHS Tayside and because the study involved medicinal products, the Medicine Healthcare Regulatory Authority (MHRA) also provided an official review of study documents, facilities, and resources.

Study Sponsor

Clinical Academics and Senior Consultants from the University of Dundee reviewed the protocol and scientific quality of the study.

Funding

The proposal directly follows on from observational research that had been peer-reviewed and accepted for fast-track publication in Thorax.[63] The observational work had identified the adverse effect with the gene configuration and indicated a need for randomised controlled trials to evaluate benefit with the two commonly available modes of treatment at this stage of asthma, specifically in the population with the gene variation. This led to this proposal for research. The

proposal was reviewed and subsequently supported by the review process of pharmaceutical company Merck, Sharpe and Dohme through an unrestricted educational grant. The work was also supported by funding from the University of Dundee Medical School, Scottish Enterprises, The Gannochy Trust, The Perth & Kinross Council and the Brighton and Sussex Medical School.

Tayside Research Ethics Committee

Once the protocol had been peer reviewed and funding provisionally secured subject to mandatory regulatory approval, the proposal was submitted to Tayside Research Ethics Committee (REC).[76] The REC viewed the study protocol and documentation to ensure the research complied with ethical standards in the interests of the potential research participants i.e. children aged 5-18 years and their families. Information leaflets for parents, and versions adapted for teenage and younger participants were checked for clarity and appropriateness for target groups (appendix 1).

Approval was withheld after the first submission, but granted after alterations to the information leaflets to include explanation about confidentiality of study information, explanation to participants that data was anonymous and stored in a password protected computer, and finally a paragraph so that participants could read clearly that they could withdraw from the study at any time. The committee also requested separate information was prepared for participants about the study medicines, how they work and potential adverse effects of the medicines.

NHS Research & Development

An application was submitted to NHS Tayside Research and Development Department to request permission to undertake research on NHS Tayside premises with its patients and staff. The Research and Development Department performed a site specific assessment i.e. the suitability of the site and facilities for the research**.**

Maternal and Child Health Sciences

Head of Department & James Mackenzie Professor of Child Health
Professor Richard E Olver BSc MB BS FRCPCH FRCPE FMedSci

Appendix 1

RESEARCH INFORMATION SHEET FOR CHILDREN (5-12 YEARS)

Title: Add-on salmeterol versus montelukast in patients with asthma carrying the Arg/Arg-16 polymorphism (Add-on asthma medicines for children with special genes)

This is an invitation to take part in a research study.

Why have I been asked?
You gave us a mouthwash sample for our Genes in Asthma study. By testing your mouthwash, we have found that you have a type of gene that will not allow one of your asthma medicines to work very well. This type of gene is present in about 1 in 8 persons. Therefore it is likely that several of your friends will have this gene also.

You may already be on these medicines or you may be given these medicines to take in next few years. Many young people are now being prescribed these medicines at an early stage in their asthma.

There is an alternative medicine (montelukast as tablet) that is not affected by the type of gene that you have, so it may be a better drug for your asthma.

We will find out which the two medicines is the right one for children like you who carry this gene. We will follow you up carefully and let you and your GP have your results at the end of the study.

So, joining in could help your asthma. It will also help us find out more about asthma in children in Scotland.

What does the study involve? THERE ARE NO BLOOD TESTS. We will ask you to take either an inhaler and a sugar tablet or an inhaler and a montelukast tablet for 1 year. You will be checked carefully over three months by a nurse and doctor who is an expert in asthma. We will also do simple and easy-to-do tests at each visit to measure how much allergy and asthma you have.
At the end of the year, we will let you and your doctor know whether the medicine has made your lung function and chest inflammation and your asthma better or worse.

Who is checking the study? Before any research is allowed to happen, it has to be checked by a group of people who make up the Ethics Committee. They make sure that the research is OK to do. This project has been checked and approved by the Tayside Ethics Committee. Taking part in the research is entirely up to you and your parents.

Who will know about my role in the study? The doctors and nurses taking part in your care will know about your role in the study. The University and the company supplying some of the medicines (MSD) may also know under special conditions. The information collected about you will be stored by using a special code instead of your name on a computer that can only be accessed by a password. Only members of the research team will know your name and all the information collected will be stored securely at Ninewells Hospital for 15 years.

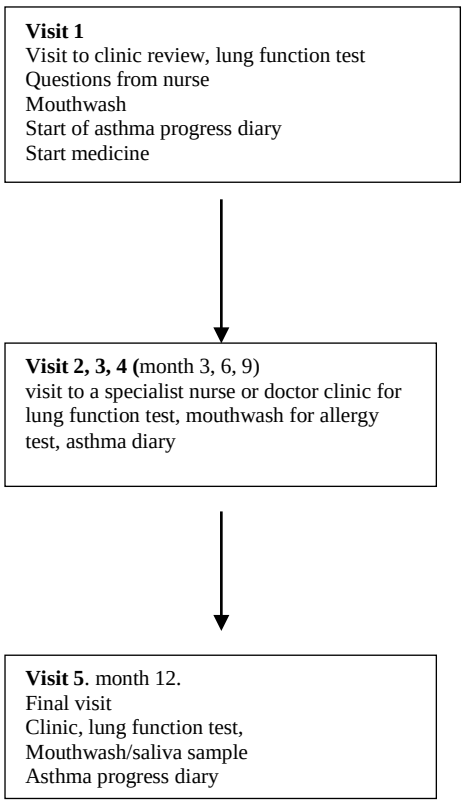
What happens if a better medicine comes along? If a better treatment comes along, taking part in this study will not stop you getting it.

Appendix 1

What if I don't want to do the research anymore? If at any time you don't want to do the research anymore, just tell your parents, doctor or nurse. They will not be cross with you. Your doctor will help you decide which medicine is best to use afterwards.

What if something goes wrong? The study only uses normal asthma drugs which are known to be safe. The nurse will be in touch with you to check that your asthma is under control. The nurse and the doctor will carefully follow up your treatment over the year and check for any problems with asthma control.

Flow chart for the study



Thank you for reading this Information Sheet and considering taking part in this study

What is your name? ……

Would you like to take part in this project? Yes …… No……...

Somnath Mukhopadhyay
Donald Macgregor
Brian Lipworth
Asthma doctors in Tayside Women's and Children's / Medical Directorate

Colin Palmer
scientist

Leaflet version 2 date 06/2007

Medicines and Healthcare products Regulatory Agency – MHRA

Clinical trials involving medicinal products require authorisation from the Medicine and Healthcare products Regulatory Agency (MHRA). An application to MHRA requires detailed information about all aspects of the trial medicines, dosing, storage and the supplier of the medicines. The Clinical Trial Pharmacist prepared an example of the labels to be used on the study tablets supplied to participants at each visit.

MHRA requested modification to the study protocol before giving approval to commence the study. The protocol was updated to include details of safety reporting procedures in accordance with the Clinical Trials Directive 2001/20/EC.[77]

Clinical Trials Registration

The trial was registered with ClinicalTrials.gov number NCT00655616.[78] This web-based resource provides participants, family members, health care professionals, researchers, and the public access to information about clinical studies both publicly and privately supported on a wide range of diseases and conditions.

Statistical Rationale:

I was not involved determining number of subjects but have some limited understanding of process involved. Before a study is conducted, researchers must determine how many subjects should be included. If too few subjects are enrolled, a study may not have enough statistical power to detect a difference. Enrolling too many patients could be unnecessarily costly or time-consuming.

The statistical analysis for the trial was based on pilot data and calculated using the software Statmate 2 for Macintosh and Graph Pad Software Incorporated (San Diego, California, US).

This package was used to determine the minimum number of subjects needed for the study in order to have sufficient statistical power to detect a treatment effect between the two study groups.

Pilot work

Pilot work in Tayside (BREATHE study)[63] showed that 85% of asthmatic children who were *Arg16* homozygous and prescribed regular salmeterol had one or more school absences over 6 months, compared to 25% in those on inhaled steroids alone (i.e. a 60% difference). This is known as the effect size. If the effect size is large between the study groups then the sample size required for the study is less and if the effect size between the study groups is small, the sample size required is large.[79]

Sample size required

A sample of 30 patients in each arm was required to show a minimal important difference of 60% in school absences over 1 year as the primary outcome for comparison between the 2 groups, to achieve at least 80% power, with error of 0.05.

Margin of Error (Confidence Interval)

The confidence interval determines how much higher or lower than the population mean you are willing to let your sample mean fall. This is usually +/- 5%

Chapter 7

STUDY DESIGN

Study Description

The study is randomised controlled (parallel design) to compare oral montelukast and inhaled salmeterol as add on therapy to inhaled corticosteroid treatment over a period of 1 year, in children aged 5-18 years with asthma carrying *Arg/Arg* 16 homozygous genotype.

Primary Hypothesis

- Children with asthma carrying the *Arg/Arg*-16 genotype have fewer school absences over a period of 1 year on oral montelukast in comparison to inhaled salmeterol

Secondary Hypotheses

- Children with asthma carrying the *Arg/Arg*-16 genotype require less rescue oral steroids over a period of 1 year on oral montelukast in comparison to inhaled salmeterol

- Children with asthma carrying the *Arg/Arg*-16 genotype have less hospital admissions over a period of 1 year on oral montelukast in comparison to inhaled salmeterol

- Children with asthma carrying the *Arg/Arg*-16 genotype have less overall asthma exacerbations (total school absence, hospital admission and need for oral steroids) over a period of 1 year on oral montelukast in comparison to inhaled salmeterol

- Children with asthma carrying the *Arg/Arg*-16 genotype require less reliever medication over a period of 1 year on oral montelukast in comparison to inhaled salmeterol

- Children with asthma carrying the *Arg/Arg*-16 genotype have higher peak expiratory flow rate and higher forced expiratory volume at 1 second over a period of 1 year on oral montelukast in comparison to inhaled salmeterol.

- Children with asthma carrying the *Arg/Arg*-16 genotype report less symptoms of cough, wheeze and dyspnoea in the morning and overnight over a period of 1 year on oral montelukast in comparison to inhaled salmeterol.

- Children with asthma carrying the *Arg/Arg*-16 genotype have improved asthma specific quality-of-life over a period of 1 year on oral montelukast in comparison to inhaled salmeterol.

Study Setting

The study took place at the Children's Asthma and Allergy Research Unit, Tayside, between August 2007 and August 2009. The pragmatic design of the study helped make taking part more appealing and convenient for families because they could be seen at their normal clinical area at Ninewells Hospital and Medical School or Perth Royal Infirmary.

Recruitment

Demographic, clinical and β_2 receptor genotype information from 546 children with physician diagnosed asthma was obtained from a previous Tayside study database known as BREATHE. Gene analysis of DNA from BREATHE study mouthwash samples determined that 154 participants were homozygous for the *Arg/Arg* 16 genotype. The BREATHE study consent form included a section requesting permission to contact the families about future research. Each of the 154 participants with the *Arg/Arg* 16 polymorphism were invited to discuss the outcome of BREATHE study analysis and consider taking part in the present study.

Inclusion criteria

Sixty two children and young adults agreed to participate in the study and fulfilled all the following criteria to take part in the study:

- physician-diagnosed asthma
- prescribed daily inhaled corticosteroid therapy.
- history of at least one of the following within the previous 12 months: school absences as a result of asthma, need for course of oral steroids, visits to General Practitioner, out-of-hours service or hospital admissions with asthma.

Exclusion criteria

Children with other significant lung disease or multi-system disease e.g. cystic fibrosis, cancer under current treatment, were excluded from the study.

Enrollment & Participant Numbers

Figure 1 describes enrollment and the number of study participants. The children with Arg/Arg16 genotype (154) were screened for eligibility. Fifty two were not eligible to participate mostly because they no longer used inhaled corticosteroids, or if they were still prescribed inhaled steroids did not meet other inclusion criteria. Forty children/families declined to participate for unspecified personal reasons.

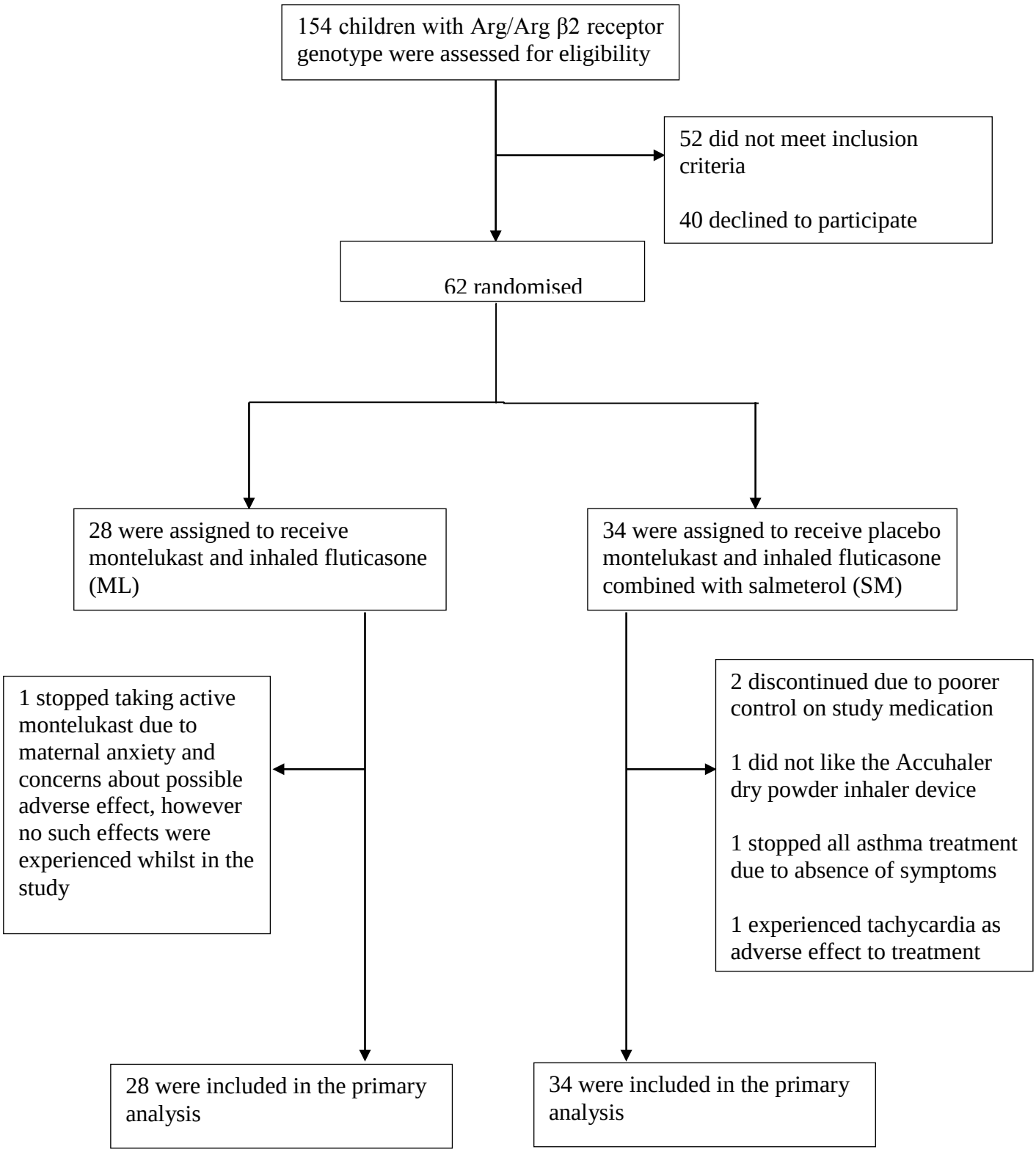
Number of Participants to complete Study

All patients for whom follow-up data was available were included in the analysis, even if they did not complete all 5 visits i.e. withdrew from the study. Twenty eight participants were randomised into one treatment group (ML). Of these 28 participants in ML group, 27 completed the study by attending five scheduled visits. Only one girl discontinued in this group due to

maternal concerns about possibly developing adverse effects from the trial medication, however no such effects were experienced by this girl whilst she was in the study.

Thirty three participants were randomised into the other group (SM). Of these 33 participants, 28 completed the study and 5 participants withdrew. Two participants decided to withdraw from the study due to perceived poorer control on study intervention treatment. One participant disliked the accuhaler dry powder inhaler device, another exercised his right to withdraw because he no longer wished to take part and the final participant withdrew from study due to perceived adverse effect to treatment – tachycardia.

Figure 1: Enrolment and Number of Participants



Informed Consent

After screening, written consent was obtained. Information about the study was sent to the families prior to initial meeting as stipulated by the REC committee. The study was explained to participants and they were given opportunity to ask questions and consider whether they would like to take part. Legally valid consent was obtained from the parents/guardians and also the agreement of school age children so both parents and children signed the consent form. Participants aged 16 years or older provided consent independently. A copy of the study consent form follows (appendix 2).

Maternal and Child Health Sciences

Head of Department & James Mackenzie Professor of Child Health
Professor Richard F Oliver BSc MB BS FRCPCH FRCPE FMedSci

Appendix 2

Add-on salmeterol versus montelukast in patients with asthma carrying the Arg/Arg-16 polymorphism

CONSENT FORM

NB. This form must be completed and signed by the research participant and/ or parent/ guardian where appropriate in the presence of someone with knowledge of the research designated by the Principal Investigator. This may be a doctor, nurse, clinical research assistant or other member of the research team who must countersign the form as witness to the participant's and/ or parent/ guardian's signature

Please tick (✓) appropriate box

Have you read and understood the participant Information Sheet? Yes ☐ No ☐

Have you been given an opportunity to ask questions and further discuss this study? Yes ☐ No ☐

Have you received satisfactory answers to all of your questions? Yes ☐ No ☐

Have you now received enough information about this study? Yes ☐ No ☐

Who have you spoken to? Dr/Mr/Mrs/Miss ……

Do you understand that your participation is entirely voluntary? Yes ☐ No ☐

Do you understand that you are free to withdraw from this study:

- At any time? Yes ☐ No ☐
- Without having to give a reason for withdrawing? Yes☐ No ☐
- Without this affecting your present or future medical care? Yes ☐ No ☐

I agree that the pharmaceutical company MSD can have access to my/my child's research and related records. Yes ☐ No ☐

Note that it is a statutory requirement that if you agree to take part in the study, your research records and, if necessary, your medical records are available for scrutiny by monitors of the sponsor organisation (which may be the NHS, University or a commercial organisation funding the study) and, in the case of clinical trials of medicines, the UK Regulatory Authorities.

Do you agree to take part in this study? Yes ☐ No ☐

Participant's signature ……Date ……..……..
Participant's name in block capital letters ……..
Parent/ Guardian's signature……Date……
Parent/ Guardian's name in block capital letters……
Telephone contact (Parent/ guardian) ……(Home)…..……(Work)
Signature witnessed by …… Date …….
Witness name in block capital letters ……..

THANK YOU for agreeing to take part in this research

Version 3; 02 July 2007

Recruitment Period & Randomisation into Treatment Groups

Participants were recruited between 1st August 2007 and 31st August 2008. The concealed web-based randomisation programme was designed by study statistician, Dr Simon Ogston. The Clinical Research Pharmacist held a copy of this to allow her to prepare trial medicinal products. After consenting to take part in the study, participants were randomly allocated to one of two treatments at the screening visit:

Treatment Groups

Treatment arm 1 – ML - Flixotide® (fluticasone propionate) via accuhaler dry powder inhaler device as per current inhaled steroid dose plus oral montelukast;

Treatment arm 2 – SM - Seretide® (salmeterol plus equivalent dose of fluticasone) via accuhaler dry powder inhaler device as per current inhaled steroid dose plus placebo montelukast.

Study medicines

Inhaled Salmeterol

Salmeterol is an inhaled long-acting beta-agonist medication recommended by British Thoracic Society/Scottish Intercollegiate Guidelines Network when symptoms are inadequately controlled on inhaled steroids.[18] Its mechanism is to relax the muscles of the small airways in the lungs and taken regularly it lasts 12 hours to keep the airways open. In combination with fluticasone propionate, the preparation is known as Seretide (GSK). Fluticasone propionate is a corticosteroid with an anti-inflammatory action that reduces swelling and irritation in the small airways.

Delivery Device

A request was made to the General Practitioner of each participant to prescribe the new inhaled medication for the treatment group i.e. Fluticasone via accuhaler device for ML group or Seretide combination treatment via accuhaler device for SM group. No effort was made to blind the prescribed inhaled medication i.e. participants could determine which group they had been allocated on collection of the new inhaler prescription. Each group of participants was exposed to only one of the study interventions i.e. parallel design (*figure 3*).

Oral Montelukast

Montelukast is a leukotriene receptor antagonist. Leukotrienes cause narrowing of the airways and inflammation in the lungs which can lead to asthma symptoms. Montelukast blocks the leukotriene pathways.

Placebo Montelukast

The purpose of using placebo for montelukast was to exclude excipient effects, and to correct for the effects of concurrent administration of tablet and inhaler on compliance of either medication in both treatment groups.

Active and placebo montelukast was prepared by the Clinical Trial Pharmacist within the hospital and a 3 month supply was dispensed to participants at each visit by Research personnel. The placebo products for montelukast 4 mg, 5 mg and 10 mg tablets were prepared and dispensed in labeled bottles in compliance with the requirements of Current Good Manufacturing Practices as specified by the European Union. Good Manufacturing Practice (GMP)[80] is that part of quality assurance which ensures that medicinal products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the

marketing authorisation (MA) or product specification. GMP is concerned with both production and quality control.

The following doses and dosage regimens were used for the study

Seretide 100 Accuhaler 1 dose twice daily plus 1 tablet daily of placebo montelukast

Seretide 250 Accuhaler 1 dose twice daily plus 1 tablet daily of placebo montelukast

Seretide 500 Accuhaler 1 dose twice daily plus 1 tablet daily of placebo montelukast

Flixotide Accuhaler 50 micrograms per blister, 1 blister dose twice daily plus 1 tablet daily of montelukast

Flixotide Accuhaler 100 micrograms per blister; 1 blister dose twice daily plus 1 tablet daily of montelukast

Flixotide Accuhaler 250 micrograms per blister; 1 blister dose twice daily plus 1 tablet daily of montelukast

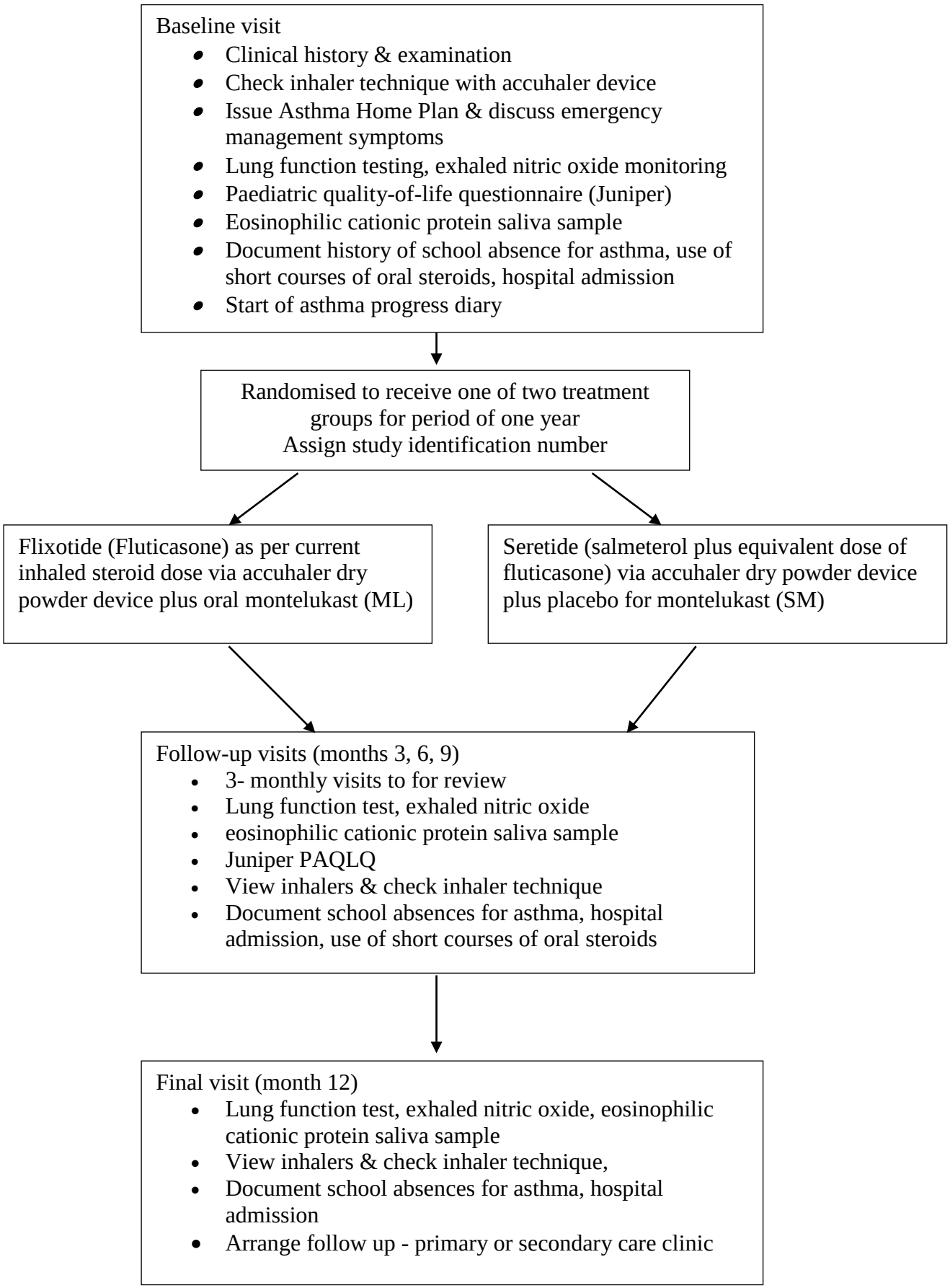
Flixotide Accuhaler 500 micrograms per blister; 1 blister dose twice daily plus 1 tablet daily of montelukast

Doses of montelukast or placebo: up to 6 years 4 mg once daily; 6-14 years 5 mg once daily; 15 years and above 10 mg once daily.

Study Visits (figure 2)

Participants were asked to remain in the study for a period of one year with five visits at three monthly intervals. On entry to the study, participant details were documented and a clinical history and examination undertaken. Each participant was designated a study identification number derived from name initials, MSD from Merck, Sharpe and Dohme who provided some of study funding and participant number randomisation e.g. AB MSD/12. A study case report form was completed at each visit. (*Appendix 3*)

Figure 2: Schematic diagram of the trial protocol



Inhaler technique

Current asthma medication was discontinued for the period of the study. The accuhaler device was chosen to deliver fluticasone and seretide because it has a dose counter which helped monitor compliance. Careful assessment of inhaler technique was necessary so that any study effect was not due to poor uptake of medication.

Patient education

Inhaled salbutamol via metered dose inhaler and volumatic device was used as rescue medication. Participants were given an individualised management plan detailing how to use salbutamol in the event of increased symptoms.

Concordance monitoring

At each visit the participant was asked to return the bottle of montelukast/placebo tablets even if there were still some tablets unused. The remaining number of tablets was documented and returned to pharmacy for disposal and a new 3 month supply of tablets was issued at each visit. The participant was asked to bring along their inhaler to allow the investigator to check total number of actuations dispsensed on the dose counter at each visit. Participant inhaler technique was also checked at each visit.

Asthma Symptom Diary

Participants were provided with an asthma symptom diary to record controller and reliever medication use and exacerbation symptoms. This was also returned every 3 months. Medication compliance review, spirometry, exhaled nitric oxide testing and safety and efficacy assessments were recorded each visit. The incidence of adverse events was recorded and serious adverse events were dealt with according to protocol as described in chapter 8.

Asthma exacerbations

Asthma-related school absences, intake of oral steroids and admission to the hospital between visits were grouped as present or absent. The total asthma exacerbation response was defined as the presence of any of these measures during the same period of time. This was again grouped as present or absent to create an asthma exacerbation yes/no response that is a valid measure of asthma severity.

Use of reliever medication

To compare the use of inhaled bronchodilator as reliever, it was classified as

0 = none used during 3 month period,

1 = occasional (more than once a week and less than daily use),

2 = daily (200micrograms/day required for symptom control),

3 = excessive use (use of more than one dose of 200 micrograms/day for symptom control).

This was then grouped as less than daily use and daily or excessive use of reliever for symptom control.

Exhaled Nitric Oxide & Pulmonary Function

Exhaled nitric oxide (Aerocrine, Solna, Sweden) and pulmonary function (forced expiratory volume at 1 second (FEV_1), forced vital capacity (FVC), peak expiratory flow rate (PEFR), forced expiratory flow between 25% and 75% of vital capacity ($FEF_{25\%-75\%}$)) (Micromed, Rochester, United Kingdom) were measured. A standard protocol was followed for spirometry.[81]

Self reported asthma symptoms

The self-reported asthma symptoms (cough, wheeze and dyspnoea at morning and over night) were classified as 0 = no symptoms, 1 = once or twice per month, 2 = once or twice weekly, 3 = daily symptoms.

RESEARCH STUDY CASE REPORT FORM - VISIT NO. _____ ***Appendix 3***

PARTICIPANT STUDY NUMBER _____

Visit Date / / / / / / / / / /

• Study No _____ Last Visit / /

• Accompanied by Mother ☐ Father ☐ Other Relative ☐
Foster Carer ☐ Social Worker ☐ Other ☐ Unaccompanied ☐

Height and Weight

Height (cm) ☐ Centile ☐ Weight (kg) ☐ Centile ☐

Medication on arrival at clinic N.B. For bronchodilator, indicate whether normal use is prn or regular.

Drug	Device	Dose per puff	Unit	Dose Prescribed	Frequency

Assessment

• Is inhaler technique satisfactory? Yes ☐ No ☐ Not on inhaler ☐ Unable to check ☐

Inhaler device viewed : Number on dose counter:

• Self-Management Plan discussed? Yes ☐ No ☐ Does not have ☐ Not applicable ☐

If the patient has a Self-Management Plan, when was it last updated? / /

• Diary/PEFR Chart seen? Yes ☐ No ☐ Does not keep Diary/PEFR chart ☐

Readings from PEFR Chart: Highest ☐ Lowest ☐

Since last visit

• Bronchodilator use None ☐ Occasional ☐ Daily ☐ Excessive ☐ Unable to answer ☐

• How many courses of oral steroids? ☐ Unable to answer ☐

• How many hospital admissions? ☐ Unable to answer ☐

• Absence from school/nursery due to asthma None ☐ 1-2 days ☐ Up to 1 week ☐
More than 1 week ☐ Unable to answer ☐ Not applicable ☐

Symptoms since last visit		None	1-2 monthly	1-2 weekly	Daily	Unable to answer
• At Night	Cough	☐	☐	☐	☐	☐
	Wheeze	☐	☐	☐	☐	☐
	Dyspnoea	☐	☐	☐	☐	☐
• Morning	Cough	☐	☐	☐	☐	☐
	Wheeze	☐	☐	☐	☐	☐
	Dyspnoea	☐	☐	☐	☐	☐

• Exercise Rarely ☐ Running ☐ Hills/Stairs ☐ Walking ☐ Unable to answer ☐

• Persistent Nasal Symptoms None ☐ Runny ☐ Sneezy ☐ Blocked ☐ Unable to answer ☐

Lung Function

	PEFR	FEV1	FVC	FEV1/FVC	F50	F25
Predicted	l/min	1	l	%	l/sec	l/sec
Actual	l/min	1	l	%	l/sec	l/sec
% Predicted	%	%	%		%	%

- Was lung function technique satisfactory? Yes ☐ No ☐

Exhaled Nitric Oxide Reading

- Was eNO technique satisfactory? Yes ☐ No ☐
- eNO reading (ppb)

Pharmacovigilance

- Any adverse events where association with trial medication suspected? Yes ☐ No ☐
- If yes details of adverse event
- Action taken

Record in Case Report Form (CRF) Yes ☐
Not required ☐
Notify Sponsor Yes ☐
Not required ☐
Report to MHRA & Ethics Yes ☐ Not required ☐
Adverse Event Report form completed Yes ☐
Not required ☐

Quality of Life Questionnaire

• Completed beginning consultation Yes ☐ No ☐

Next visit Signature

Asthma Specific Quality of Life

To measure quality of life, the Juniper Paediatric Asthma Quality of Life questionnaire was used (appendix 4). Validated for children aged 7-17years, it is designed to reflect both physical and emotional areas of daily life that children with asthma identified as being important and bothersome to them. It can show small changes even when the clinical state is stable.

Domains

The items are in three domains:

- Symptoms (n = 10)

  e.g. How much did COUGHING bother you in the past week?

- Emotional function (n = 8)

  e.g. How often did you feel DIFFERENT OR LEFT OUT because of your asthma during the past week?

- Activity Limitation (n = 5)

  e.g. How much have you been bothered by your asthma in PHYSICAL ACTIVITIES(such as running, swimming, sports, walking uphill/upstairs and bicycling) during the past week?

Appendix 4

PAEDIATRIC ASTHMA QUALITY OF LIFE QUESTIONNAIRE

I want you to tell me how much you have been bothered by your asthma during the past week. I will tell you which card to use. Pick the number that best describes how much you were bothered by your asthma during the past week.

1. (A) How much have you been bothered by your asthma in **PHYSICAL ACTIVITIES** (such as running, swimming, sports, walking uphill/upstairs and bicycling) during the past week? [BLUE CARD]

2. (A) How much have you been bothered by your asthma in **BEING WITH ANIMALS** (such as playing with pets and looking after animals) during the past week? [BLUE CARD]

3. (A) How much have you been bothered by your asthma in **ACTIVITIES WITH FRIENDS AND FAMILY** (such as playing during school break and doing things with your friends and family) during the past week? [BLUE CARD]

4. (S) How much did **COUGHING** bother you in the past week? [BLUE CARD]

5. (E) How often did your asthma make you feel **FRUSTRATED** during the past week? [GREEN CARD]

6. (S) How often did your asthma make you feel **TIRED** during the past week? [GREEN CARD]

7. **(**E**)** How often did you feel **WORRIED, CONCERNED, OR TROUBLED** because of your asthma during the past week? [GREEN CARD]

8. (S) How much did **ASTHMA ATTACKS** bother you during the past week? [BLUE CARD]

9. (E) How often did your asthma make you feel **ANGRY** during the past week? [GREEN CARD]

10. (S) How much did **WHEEZING** bother you during the past week? [BLUE CARD]

11. (E) How often did your asthma make you feel **IRRITABLE (grumpy*)** during the past week? [GREEN CARD] (*use only if patient does not understand the word "irritable")

12. (S) How much did **TIGHTNESS IN YOUR CHEST** bother you during the past week? [BLUE CARD]

13. (E) How often did you feel **DIFFERENT OR LEFT OUT** because of your asthma during the past week? [GREEN CARD]

14. (S) How much did **SHORTNESS OF BREATH** bother you during the past week? [BLUE CARD]

15. (E) How often did you feel **FRUSTRATED BECAUSE YOU COULDN'T KEEP UP WITH OTHERS** during the past week? [GREEN CARD]

16. (S) How often did your asthma **WAKE YOU UP DURING THE NIGHT** during the past week? [GREEN CARD]

17. (E) How often did you feel **UNCOMFORTABLE** because of your asthma during the past week? [GREEN CARD]

18. (S) How often did you feel **OUT OF BREATH** during the past week? [GREEN CARD]

19. (A) How often did you feel **YOU COULDN'T KEEP UP WITH OTHERS** because of your asthma during the past week? [GREEN CARD]

20. (S) How often did you have trouble **SLEEPING AT NIGHT,** because of your asthma, during the past week? [GREEN CARD]

21 (E) How often did you feel **FRIGHTENED BY AN ASTHMA ATTACK** during the past week? [GREEN CARD]

22. (A) Think about all the activities that you did in the past week. How much were you bothered by your asthma doing these activities? [BLUE CARD]

23. (S) How often did you have difficulty taking a **DEEP BREATH** in the past week? [GREEN CARD]

DOMAIN CODE:

(S) = Symptoms
(A) = Activity Limitation
(E) = Emotional Function

Format

There are 23 questions using words that children themselves may use to describe their problems. The response options are on a seven point scale where 1 indicates maximum bother and 7 no bother. The participants were given a card on which the responses were listed with a number and a description (appendix 5)

Individual items within the PAQLQ were equally weighted and results were expressed as the mean score per item for each of the domains as well as for overall quality of life. The scores were distributed as follows: symptom scores: range 10-70, activity limitation scores: range- 5- 35, emotional function scores: range- 8- 56. Higher scores indicate better quality of life and 0.5 is the minimal clinically important difference.[40]

The interviewer-administered version was used where Research Fellow or Nurse asked each question using the exact wording of the Juniper questionnaire, and the child answered with a number or phrase response from the answer card that best described his or her experiences during the previous week. Participants were asked to answer without any help or direction from their parents or the researcher.

Appendix 5

1. ALL OF THE TIME
2. MOST OF THE TIME
3. QUITE OFTEN
4. SOME OF THE TIME
5. ONCE IN A WHILE
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

1. EXTREMELY BOTHERED
2. VERY BOTHERED
3. QUITE BOTHERED
4. SOMEWHAT BOTHERED
5. BOTHERED A BIT
6. HARDLY BOTHERED AT ALL
7. NOT BOTHERED

Chapter 8

PHARMACOVIGILANCE & MONITORING OF ADVERSE EVENTS

An important aspect of clinical trial work is understanding pharmacovigilance and procedures to monitor adverse events that occur especially when the trial involves medicines. In this chapter I discuss pharmacovigilance in the trial and the safety reporting during the trial.

Pharmacovigilance and safety reporting:

Any untoward medical occurrence affecting the participants during the course of the trial, referred to an adverse event, must be reported in accordance with the Medicines for Human Use (Clinical Trials) Regulation 2004[82] e.g. one participant was admitted to hospital for an unrelated incident during the trial and in accordance with local guidelines a form was completed to inform Research Ethics department. No further action was required in this event because no serious event happened to participant and the reason for hospital admission was unrelated to the trial medicines.

Definitions of Other Possible Events:

Serious Adverse Event (SAE)

A SAE is an adverse event where the participant has died or where a participant was admitted to hospital over a prolonged period, or if a participant had sustained persistent or significant disability or incapacity.

There were no SAE's in the trial. The definition of an SAE required us to consider that other adverse events may fall into the 'serious' category if they were important medical events (other than those listed in definition above) e.g. requiring medical investigation or intervention.

Adverse Reaction (AR)

An adverse reaction is when there is at least a possibility that the cause of an event is linked to the trial medicine.

Serious Adverse Reaction (SAR)

A serious adverse reaction (SAR) is a serious adverse event (SAE) that is thought to be linked to the trial medicine or intervention.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious unexpected serious adverse reaction (SUSAR) is defined as an unexpected occurrence of a SAR. This could be a previously unreported reaction to a trial medicine, or a previously reported but worsening or unexpectedly frequent adverse medicine reaction.

Procedure for Recording and Notification:

Participants and their family were asked if they had experienced any symptoms or events that may have occurred since their previous visit. An assessment was made of causality and recorded this in the case report form (CRF). There were 7 adverse events during the trial period. Fortunately none of these were considered to be in the serious category.

If the events had fallen into the serious category where the event may have or had the potential to affect their continued participation in the trial, the study sponsor, University of Dundee, would have been notified. It was not felt necessary to notify the study sponsor of admissions to hospital with acute asthma that can be anticipated with the natural course of the disease. The investigator did not notify the sponsor of any serious adverse reaction which was already described in the summary of product characteristics for the study drugs, montelukast and seretide, unless it affected or had the potential to affect the continued participation of the subject in the study.

The procedure for notification was laid down as follows. The investigators notified SAEs or SAEs at the earliest convenient time after the investigator became aware of the event. The immediate report provided a limited amount of detail, including the subject's unique identifier, gender and age plus a brief statement of the event. For this purpose, there is a dedicated email address is provided by NHS Tayside, pharmacovigilance.tayside@nhs.net . A detailed written report followed, containing all the information required by the sponsor for onward reporting to the Regional Ethics Committee. This would have also included MHRA if there had been an adverse reaction during the trial.

Study Adverse Events

- Participant no. 2 admitted to short stay ward Ninewells due to intoxication from alcohol and possible drug ingestion

- Participant no. 48 randomised into placebo group (seretide 125mcgs b.d.). Mother felt him to be hyperactive and he reported palpitations. Parent made decision to stop treatment (withdraw from study) & not to see G.P. or Research staff to discuss

- Participant no. 58 developed coryzal symptoms. Saw G.P. no change to treatment. Cough began again. Saw G.P. 9/04/08 questioned whether related to new accuhaler. Participant decided to withdraw from study

- Participant no. 38 admitted to ward 29 Ninewells Hospital due to acute asthma. Treated with oral steroids

- Participant no. 38 readmitted to ward 29 Ninewells Hospital due to ongoing asthma symptoms

- Participant no. 6 required oral steroids and antibiotics due to worsening asthma. Feels quality of life worsened since stopping pre-trial medicines

- Participant no. 31 admitted to ward 29 Ninewells due to acute asthma. Treated with oral steroids & nebulised bronchodilators

Risk Assessment of medicinal products:

The investigation of medicinal products used in the study, montelukast and seretide, were used in their licensed dosage forms. Both drugs have been widely used in the treatment of childhood asthma for over 10 years. Their side effect and toxicity profiles are therefore already well-established in the study population.

Chapter 9

STATISTICAL METHODS

Statistical analysis

I was involved in collection of the data and entered data onto the results database in preparation for analysis. I did not directly carry out the analysis but have tried to gain an understanding of the process of analysis of data in a clinical trial. All participants from both groups were included in the intention-to-treat analysis. No interim analysis was performed before completion of the trial.

Software Packages

The analysis was performed using the Statistical software Package for the Social Sciences referred to as SPSS. This is compatible with Windows version 16 (SPSS Inc, Chicago, Ill). Prism (GraphPad Software Inc) software package was used to analyse and present the data in graphs.

Baseline calculations

Means (Standard Deviations) were calculated for baseline variables. Comparisons were made by an overall analysis of variance, and Bonferroni-corrected, multiple-range testing.[79]

Means (Standard Deviations)

Bonferroni-corrected, multiple-range testing

Bonferroni correction, named after Italian mathematician Carlo Emilio Bonferroni, is a statistical method used to counteract the problem of multiple comparisons. [83]

Comparisons

Comparisons refer to the 2 treatment groups. Efficacy was compared between the two treatment groups, montelukast and salmeterol, in terms of the reduction of any one of a number of disease symptoms. Where more symptoms are considered, it becomes more likely that there will appear to be an improvement in one medicine or the other in at least one of the symptoms. Multiple comparisons arise when a statistical analysis encompasses a number of comparisons.

For the study means (SDs) were calculated as descriptive statistics for baseline variables. Comparisons were made by an overall analysis of variance, followed by Bonferroni-corrected, multiple-range testing, to obviate multiple pair wise comparisons between baseline and the two treatment groups, with the overall error set at $P<.05$. A mixed-effects linear model for longitudinal data analysis was used as we had obtained repeated measurements of the primary and secondary outcome variables over time. Outcome variables based on daily symptoms and diary records were averaged over all the days between clinic visits.

Mixed-effects linear model for longitudinal data analysis

Data generated by observing a number of individuals repeatedly under differing experimental conditions where the individuals are assumed to constitute a random sample from a population of interest.[83] Our study was designed to compare mean responses over time among 2 groups of individuals. For balanced and complete data, a generalized multivariate analysis of variance is used (ANCOVA).

Assessing normality of data using parametric testing

When choosing analysis or which statistical test to perform it is important to determine whether you have data that allows for parametric or non-parametric testing. The SPSS package allows the user to assess whether their data is normal. Parametric testing assumes that the data is normal. Parametric testing uses more information than non-parametric tests and are therefore usually more powerful.[83]

Probability (P)

The probability (p) value indicates how likely it is that the result of the study could have arisen by chance alone. Results were considered significant if the probability was 0.05 or less.[79]

Confidence intervals

Confidence intervals were used because the P value does not tell us about the size of the effect. Confidence intervals tell us how much the play of chance might have altered the result. Chance variation becomes smaller and the confidence interval narrower where more participants are studied.[79,83]

Error bars

Error bars represent 95% confidence intervals.[83] *P* values are shown for the comparison between the two treatment groups through the treatment period.

SECTION 3 - RESULTS

Chapter 10

In this chapter I will to display study results. The first chapter provides a summary of baseline characteristics of participants obtained at entry to the study. Following this there is a short description of the results obtained for each outcome in the study and a graph to illustrate the result.

BASELINE CHARACTERISTICS

It is important to show that any differences between the study groups at start of the trial was not a factor in the overall analysis. A comparison of the baseline characteristics of study participants in each group was made. The results are divided into two treatment groups – inhaled Fluticasone and montelukast (ML) and inhaled Fluticasone with salmeterol and placebo montelukast (SM) to show asthma control in each group over the previous year prior to entry into the study. Overall participants in both groups had well controlled asthma.

Age in years; study population; body mass index at baseline

Mean age in years of participants at randomisation was calculated by dividing the sum total of ages in each group with the number of participants in each group. Participants in the SM group were slightly older (11.79 years) compared to 10.50 years for the ML

group. The study population is similar to the general population of children with asthma where the prevalence of asthma in boys known to be greater than in girls.[85] In each treatment group the percentage of boys is 56% and 71%. Both groups were within normal range for body-mass index (weight in kilograms divided by the square of the height in meters). None of these differences were significant at baseline.

Asthma control prior to entering study

The degree of asthma control at baseline was assessed by recording how frequently participants required short acting beta agonists over the three month period prior to entering the study. A high percentage, 96 % in ML and 91% in SM of participants reported using salbutamol at least twice weekly at baseline.

Participants were asked if they had required any short courses of oral steroids, had asthma-related school absences, or had any exacerbations resulting in hospital admission within the year prior to entering the study. A high number of participants in each group reported school absences due to asthma – 96% in ML group and 92% in SM group. School absence was one of the inclusion criteria for the study. 22% of ML and 15% of SM group reported an asthma exacerbation requiring admission to hospital within the previous year. None of these differences were significant at baseline.

There was a significant difference in pre-treatment requirement of oral steroids due to asthma exacerbations in the previous year ($p = 0.011$) between the two treatment groups. This could have suggested this group had more severe asthma at baseline and more

potential room for improvement, compared to the salmeterol group. To demonstrate that this difference was not a factor in the overall analysis the ANCOVA model was used.

ANCOVA analysis

ANCOVA (analysis of co-variance) analysis allows researchers to demonstrate they have considered the statistical effect initial differences (in this study oral prednisolone use) and provide as accurate picture as possible that this did not affect overall effect of the two treatments.[83]

Baseline Pulmonary function: Peak expiratory flow rate (PEFR); Forced expiratory volume in one second (FEV_1); Forced vital capacity (FVC) at baseline

All participants in both groups were able to perform baseline pulmonary function measurements.

Asthma scores from Paediatric Asthma Quality of Life Questionnaire (PAQLQ) at baseline

The Juniper Paediatric Asthma Quality of Life questionnaire was used to show how participants perceived their asthma symptoms and the effect of asthma on their every day life. Higher scores indicate better quality of life and 0.5 is the minimal clinically important difference. Pre-treatment mean PAQLQ symptom scores of 5.67 (ML) and 5.52 (SM), activity limitation scores of 5.71 (ML) and 5.71 (SM), and emotional function scores of 6.09 (ML) and 5.91 (SM) were observed. None of these differences were significant at baseline.

Self reported asthma symptoms at baseline

Participants and/or their parents were asked to summarise the frequency with which they were troubled by cough, wheeze and dyspnoea both overnight and in the morning, over the 3 months before entering the study. Overall a larger percent of participants about to enter the ML treatment group reported most asthma symptoms of cough, wheeze and dyspnoea. However, these differences did not reach significance at baseline.

Daily inhaled corticosteroids & modified British Thoracic Society (BTS) step of asthma treatment at baseline

All participants had been prescribed inhaled corticosteroids at the point of entry to the study. This was also criteria for entry in the study. The level of asthma severity according to the British Thoracic Society stepwise guidelines was recorded. The number of participants at step 2 treatment i.e.on regular inhaled steroids and inhaled β_2 agonists as and when required was very similar (39% in ML and 41% in SM). There were fewer participants at step 3 in the ML group (11%) compared to 35% in SM, (i.e.prescribed step 2 treatment with addition of inhaled long acting β_2 agonists). 50% in ML group and 24% in SM were at step 4 (Step 3 treatment and montelukast). None of these differences were significant at baseline.

Chapter 11

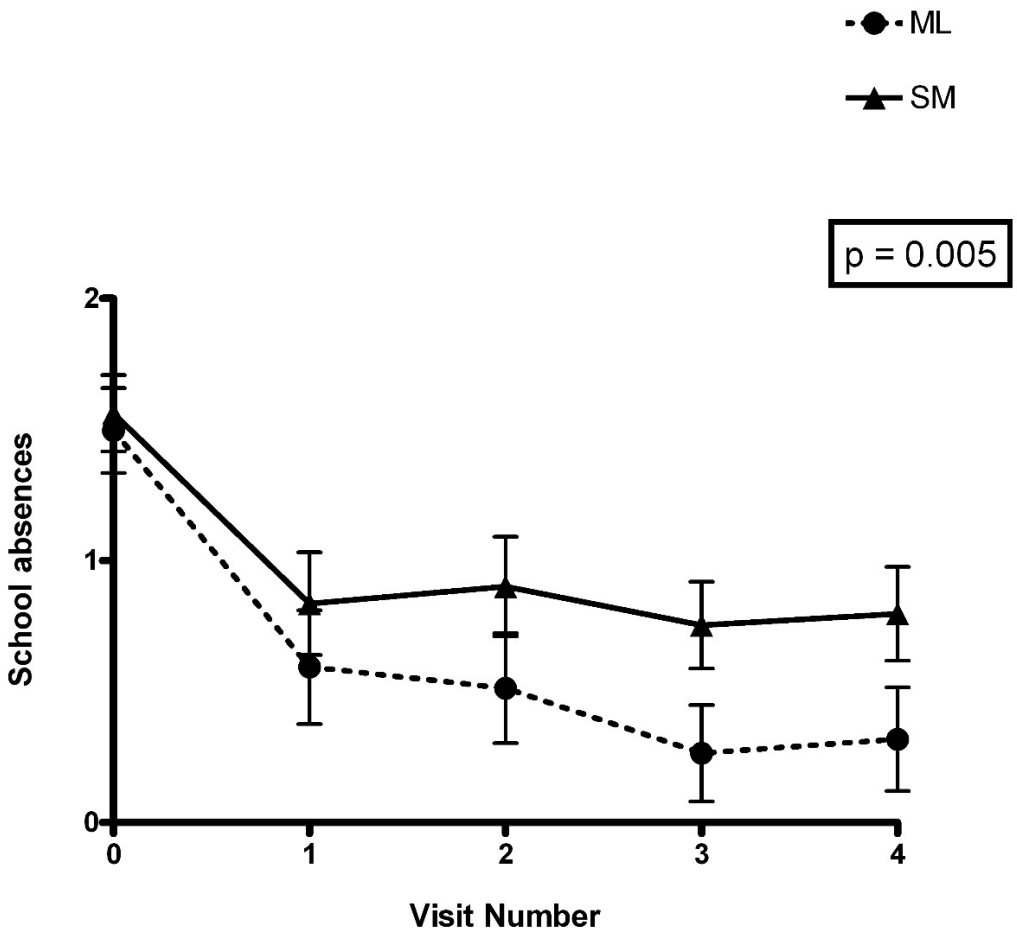
COMPARISON OF END OF STUDY OUTCOMES

In this chapter I have prepared a graph for each outcome of the study. Results are divided into two treatment groups – inhaled Fluticasone and montelukast (ML) and inhaled Fluticasone with salmeterol and placebo montelukast (SM). Each figure describes the comparison made at each visit over the one year period of study. Error bars represent 95% confidence intervals and the p value is shown.

Exacerbations

The number of episodes of asthma-related school absences, requirement of short courses of oral steroids and the occurrence of exacerbations over the study period between the two treatment groups were compared. A significant difference was observed between the treatment groups for the primary outcome of the study, asthma-related school absences (0.40 (95% CI, -0.07-0.87; p=0.005)) (figure 3). No significant difference was observed for requirement of one or more courses of oral steroids (0.13 (95% CI, -0.07-0.34; p=0.202) (figure 4) and hospital admissions (0.03 (95% CI, -0.09-0.02; p=0.241) (figure 5) during the study period. However, when considering total exacerbations (including all 3 of the above individual measures), there was a significant difference over the study period in the ML compared to SM (0.39; 95% CI, -0.20-0.99; p=0.049) (figure 6).

Figure 3: Change in asthma-related school absences



7

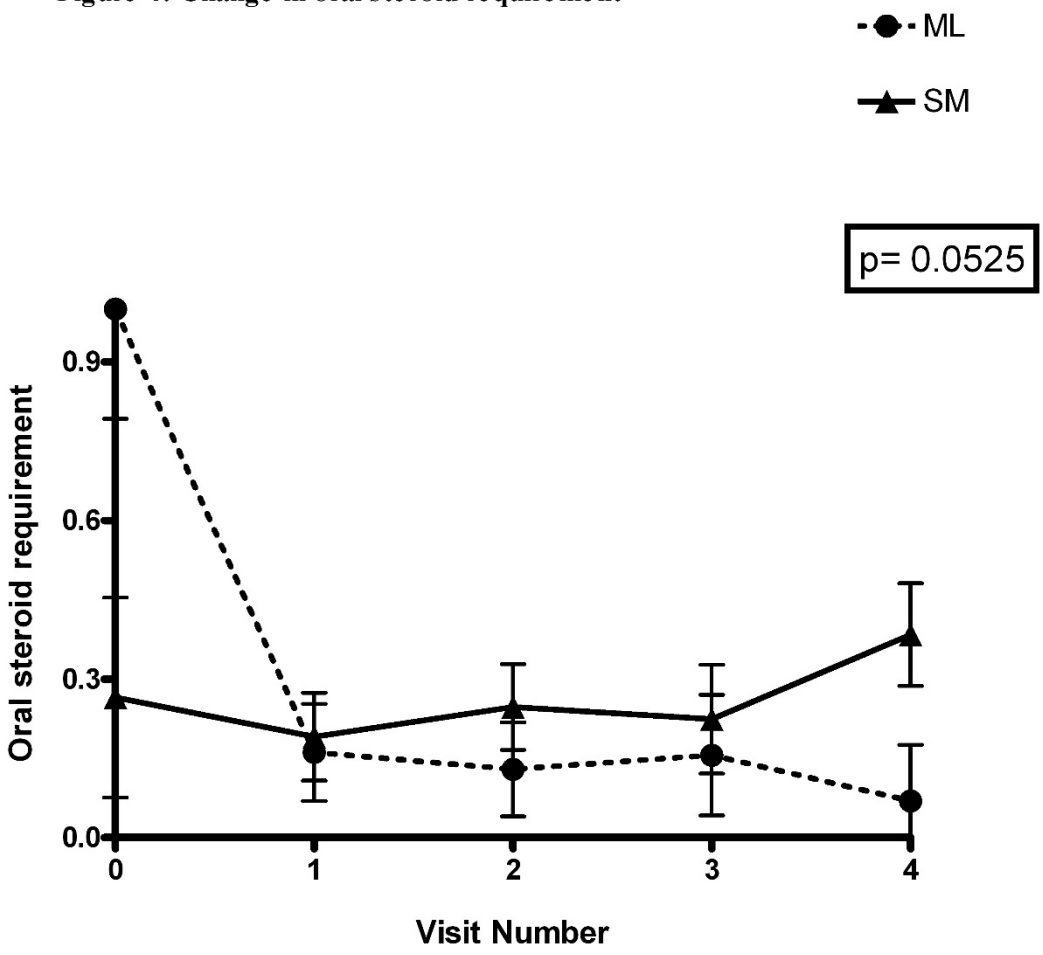
Comparison of mean asthma related school absences while assigned to receive treatment with fluticasone plus montelukast (ML) or fluticasone plus salmeterol with placebo montelukast (SM). Participants and/or their parents reported the number of school absences at 3 monthly follow-up visits (1 – 4).

0 = no school absence;
1 = 1-2 days school absence;
2 = up to one week school absence.

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups.

** Significant differences were observed (p = 0.005). Participants in the ML group had fewer school absences in comparison to the SM group.*

Figure 4: Change in oral steroid requirement

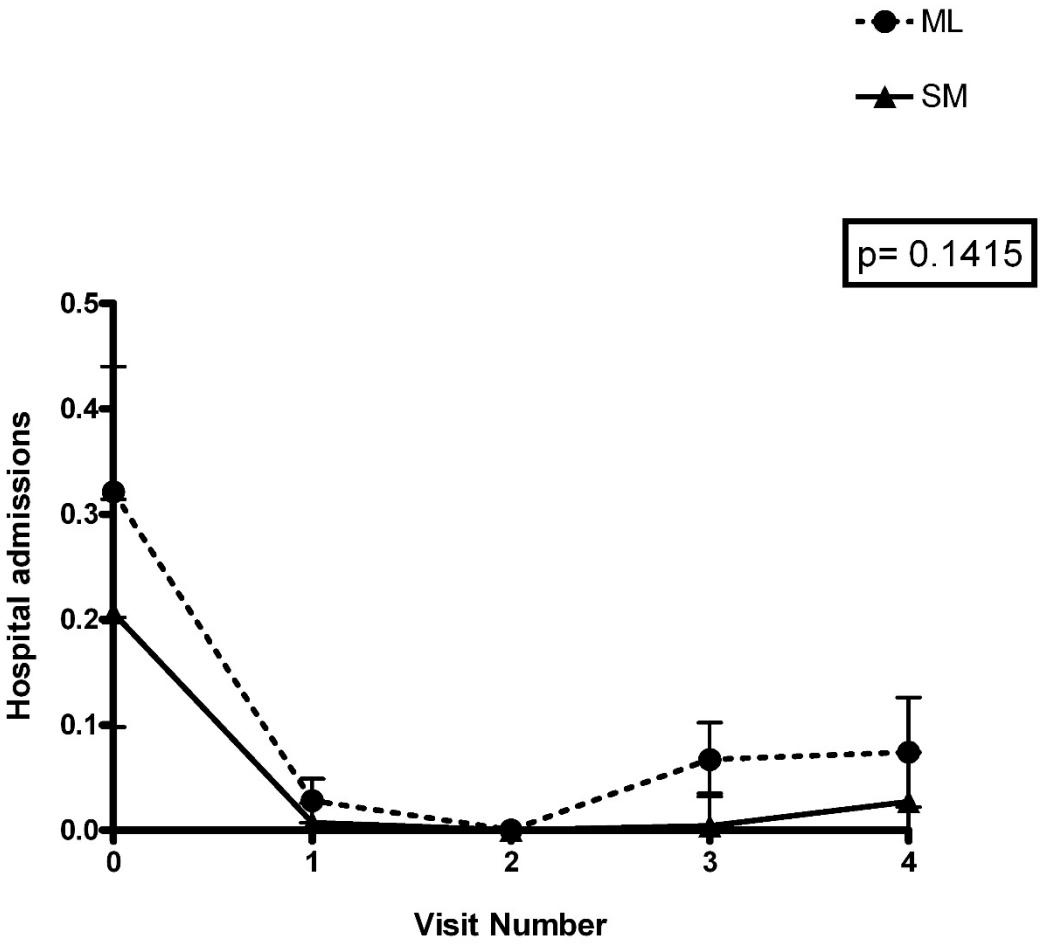


Comparison of the mean number of courses of oral steroids required to control exacerbations while assigned to receive treatment with fluticasone plus montelukast (ML) or fluticasone with salmeterol and placebo montelukast (SM). Participants and/or their parents reported at 3 monthly follow-up visits (0 – 4) during study period of 12 months.

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups.

No significant difference was observed between the treatment groups in the individual measures for requirement of one or more courses of oral steroids (p = 0.0525).

Figure 5: Change in asthma-related hospital admissions

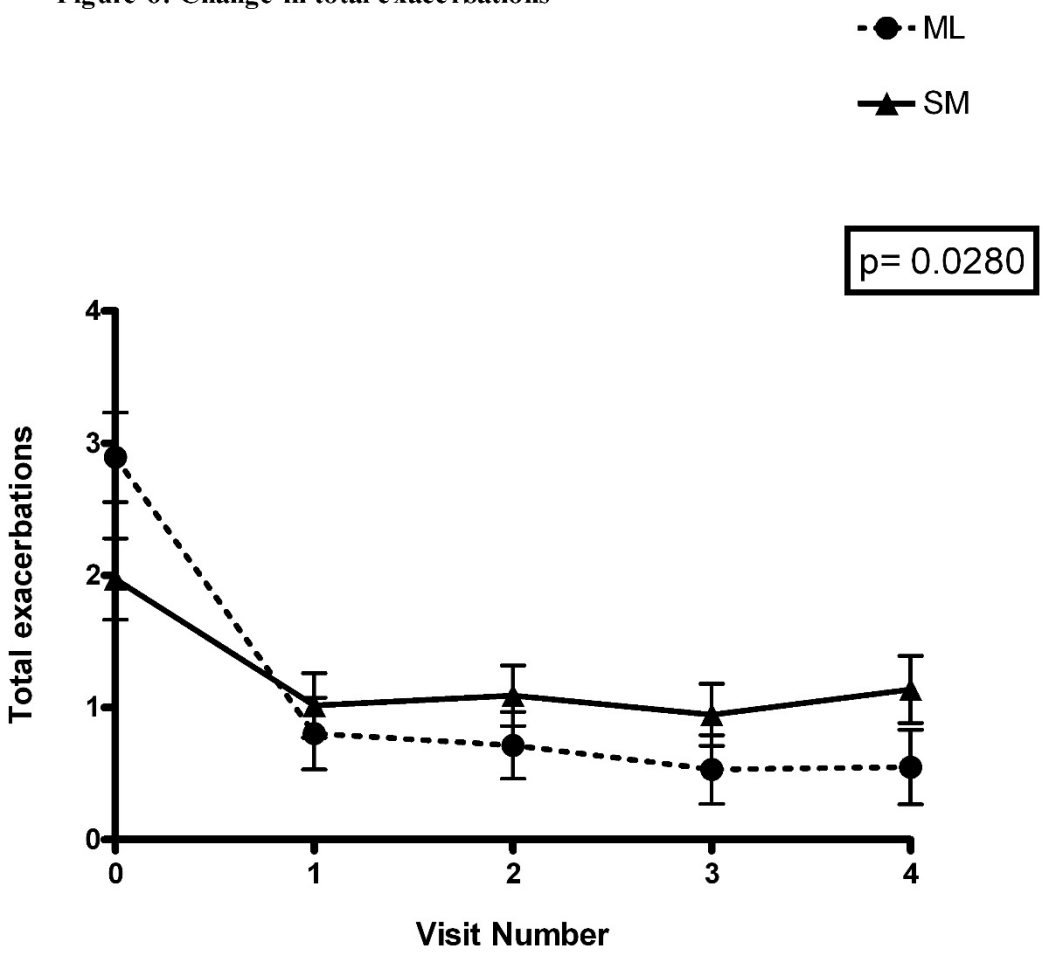


Comparison of mean asthma related hospital admissions while assigned to receive treatment with
fluticasone plus montelukast (ML), or fluticasone with salmeterol and placebo montelukast (SM), reported at 3 monthly follow-up visits (0 – 4) during the study period of 12 months.

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups.

No significant difference was observed between the treatment groups in the individual measures for hospital admissions (p = 0.1415).

Figure 6: Change in total exacerbations



Comparison of mean total exacerbations defined as either school absences, courses of oral steroids, or hospital admissions while assigned to receive treatment with fluticasone plus montelukast (ML), or fluticasone with salmeterol and placebo montelukast (SM). Participants and/or their parents reported if child had experienced any exacerbations at 3 monthly follow-up visits (0 – 4) during the study period of 12 months.

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups.

** Significant differences were observed (p = 0.0280). Participants in the ML group had fewer exacerbations in comparison to the SM group.*

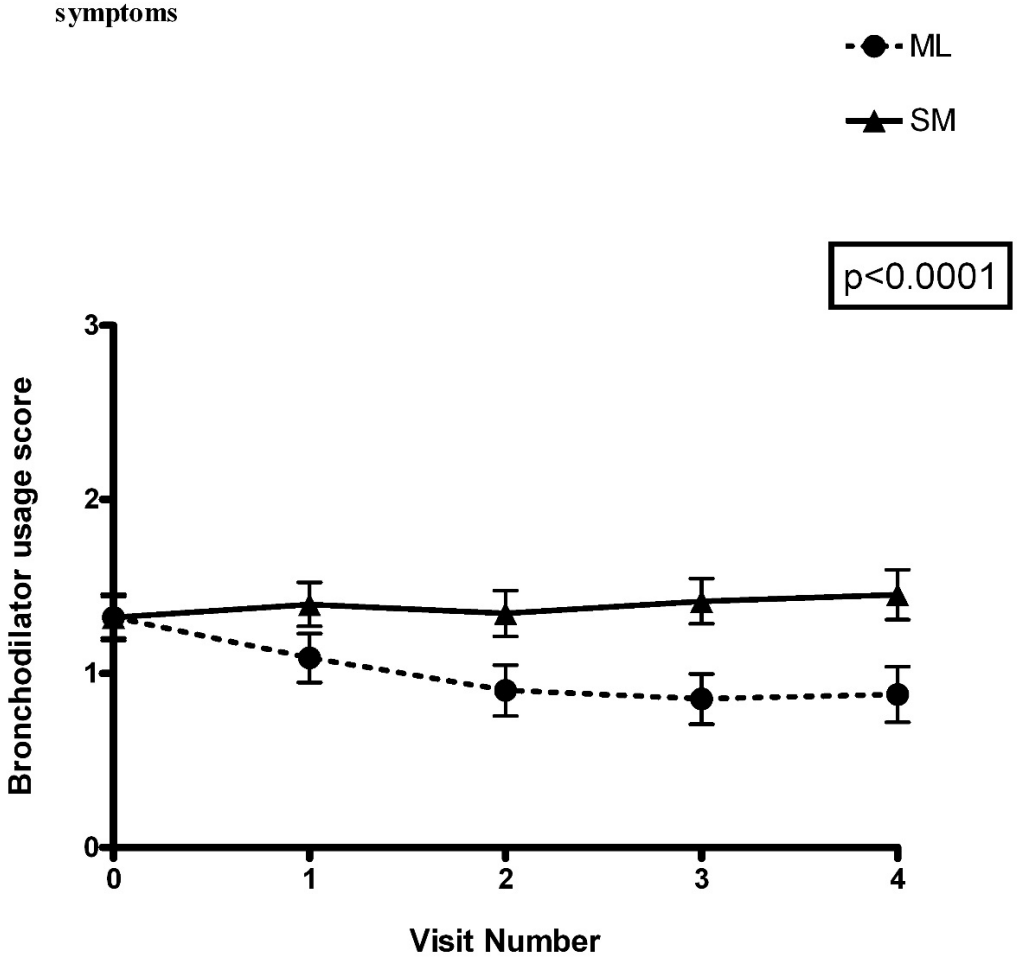
Chapter 12

COMPARISON OF END OF STUDY OUTCOMES

Bronchodilator use and Pulmonary function

The use of reliever medication was significantly decreased in ML compared to SM (figure 7). During the study period, daily or more frequent use of short acting β_2-agonist as reliever in the participants in the SM group did not alter over time (baseline 32%, 3months 38%, 6months 32%, 9months 38%, 12months 35%). However, in ML, the requirement for daily or more frequent inhaled short acting β_2-agonists was reduced over the study period (baseline 36%, 3months 18%, 6months 14%, 9months 11%, 12months 18%).

Figure 7: Change in the use of reliever medication for control of asthma symptoms



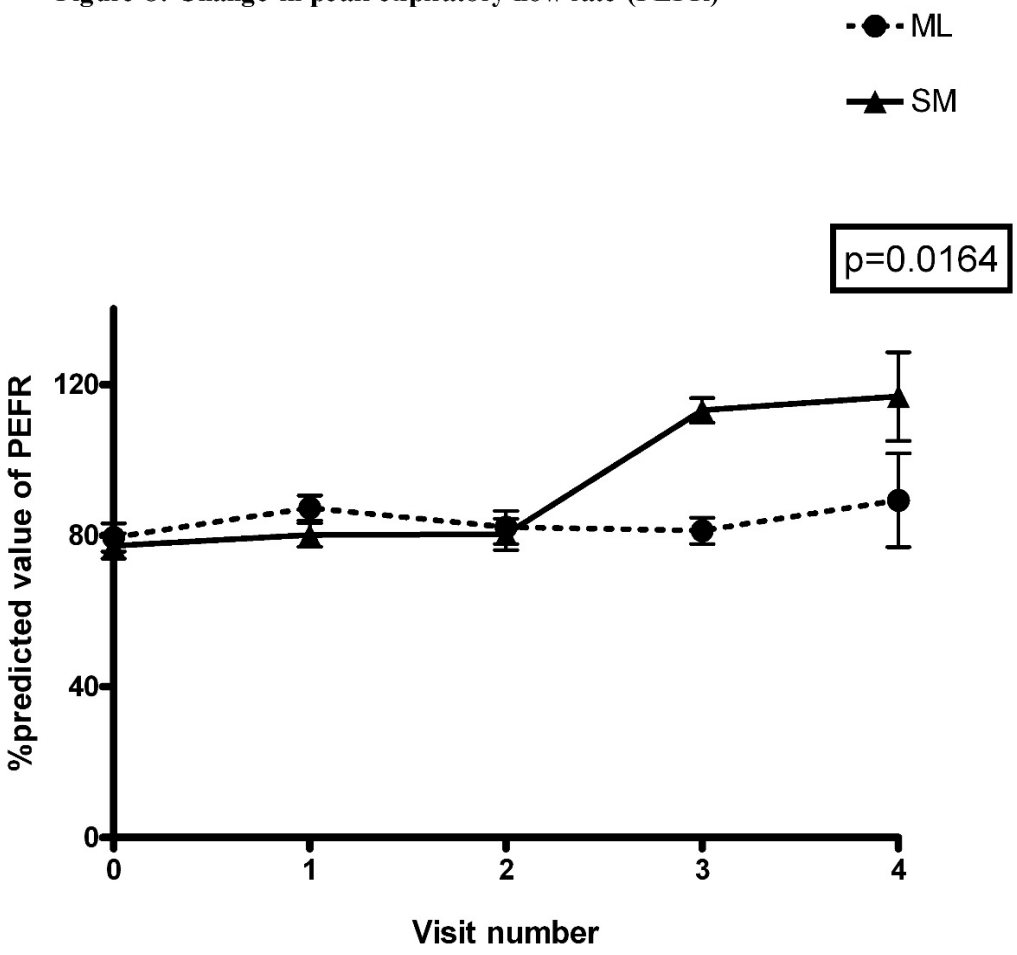
Comparison of use of inhaled bronchodilator medication for control of asthma symptoms between treatment groups - fluticasone plus montelukast (ML) and fluticasone and salmeterol with placebo montelukast (SM). Participants and/or their parents reported how much bronchodilator inhaler they had required at baseline and 3 monthly follow-up visits (0 – 4) during the study period of 12 months.

0 = no reliever medication used,
1 = occasional reliever medication used (more than once a week and less than daily use)
2 = daily (200micrograms/ day required for symptom control).

Error bars represent 95% confidence intervals and the plots represent the mean score for each group.

** Significant differences were observed (p< 0.0001). Participants in ML group used less bronchodilator than in SM group.*

Figure 8: Change in peak expiratory flow rate (PEFR)

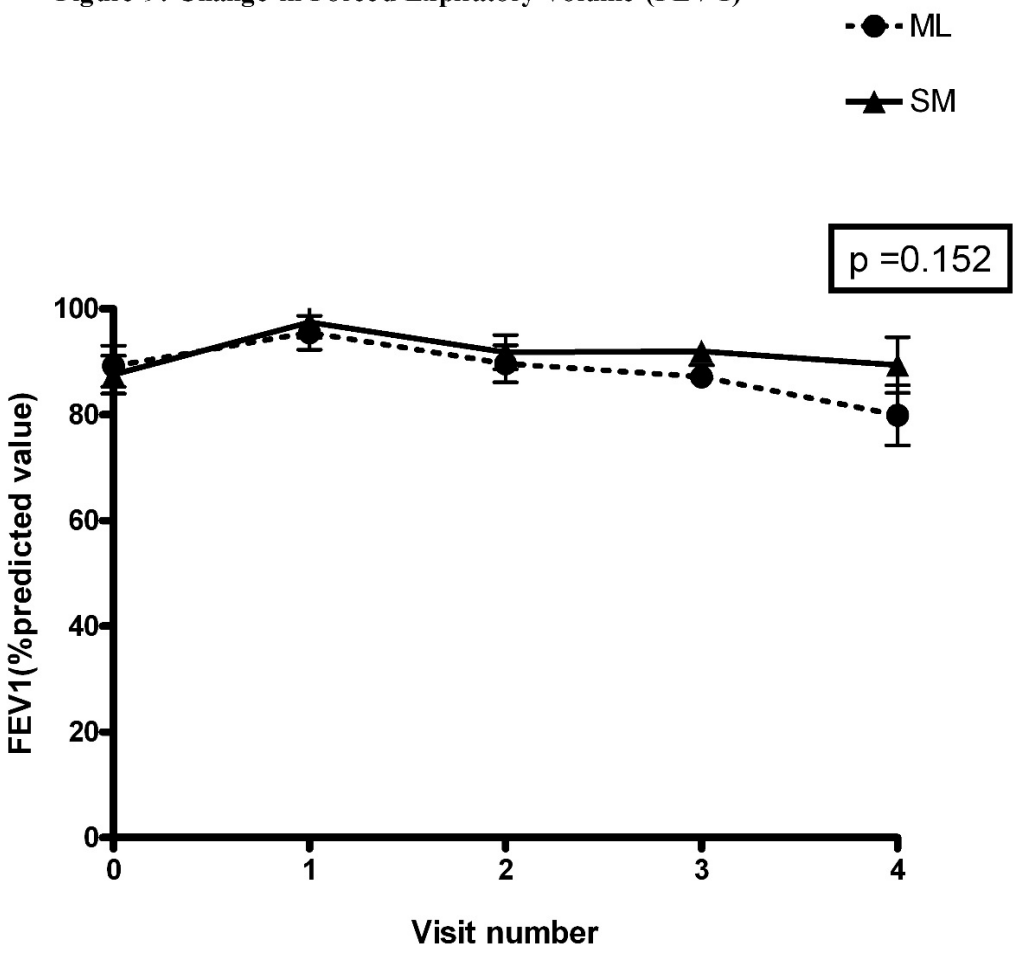


Comparison of mean values of peak expiratory flow rate (PEFR) readings shown as percent predicted (Zapletel) between two treatment groups - fluticasone plus montelukast (ML) and fluticasone with salmeterol and placebo montelukast (SM). This was recorded at baseline and 3 monthly follow-up visits (0 – 4) during study period of 12 months.

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups through the 12 month treatment period.

No significant differences were found between the groups (p=0.164).

Figure 9: Change in Forced Expiratory Volume (FEV 1)



Comparison of mean values of forced expiratory volume in one second (FEV1) readings shown as percent predicted (Zapletel) between two treatment groups - fluticasone plus montelukast (ML) and fluticasone with salmeterol and placebo montelukast (SM). This was recorded at baseline and 3 monthly follow-up visits (0 – 4) during study period of 12 months.

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups.

No significant differences were found between the groups (p=0.152).

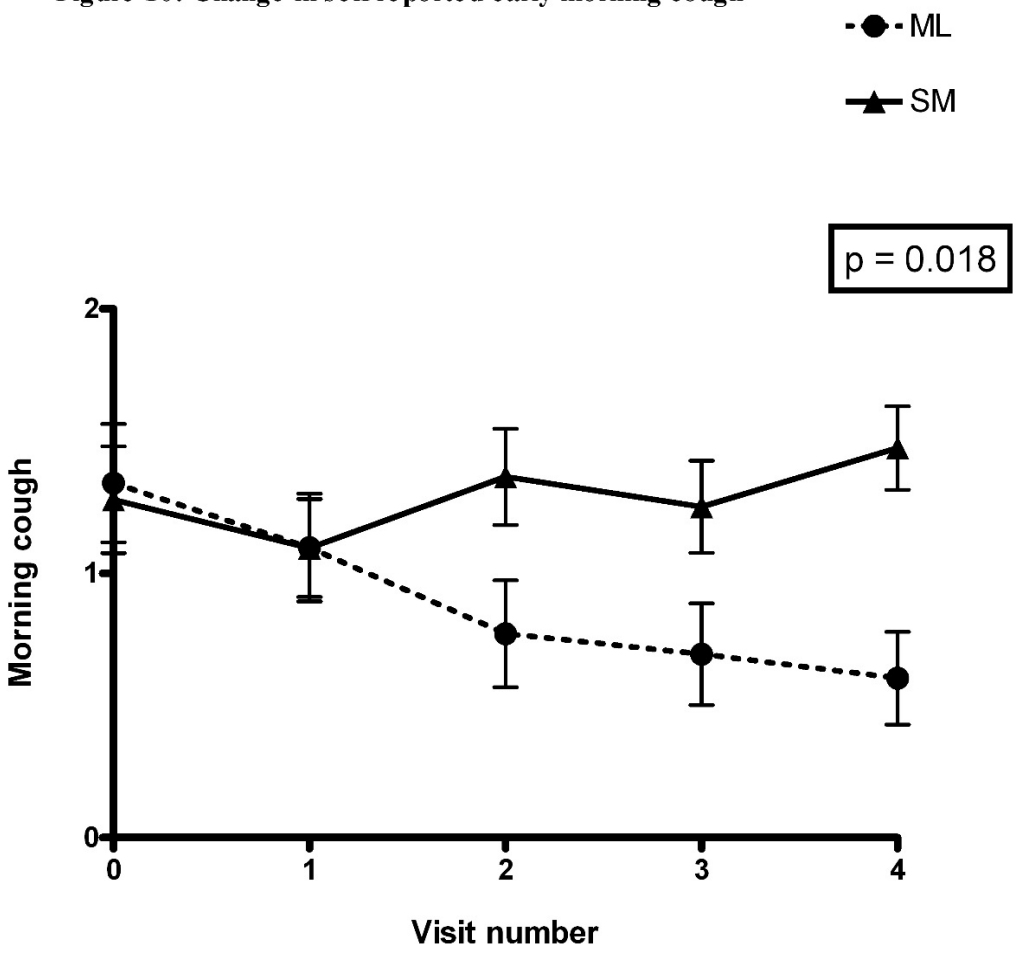
Chapter 13

COMPARISON OF END OF STUDY OUTCOMES

Parent or Self reported symptoms of Cough, Wheeze or Dyspnoea

Through the study period, the self reported symptoms of cough, wheeze and dyspnoea improved significantly in ML group compared to SM. In particular, early morning symptoms were significantly improved in ML (morning cough 0.51 (95%CI, 0.09-0.92; p=0.018) (figure 10); morning wheeze 0.55 (95% CI, 0.25-0.86; p=0.001) (figure 11); morning dyspnoea 0.29 (95% CI, 0.06-0.53; p=0.008) (figure 12)). There was no significant difference in night cough between the treatment groups (0.29, (95% CI, -0.06-0.63; p=0.238)) (figure 13). However, wheeze and dyspnoea at bedtime were improved in ML compared to SM (night wheeze 0.46 (95% CI, 0.15-0.77; p=0.004) (figure 14), night time dyspnoea 0.44 (95% CI, 0.16-0.73; p=0.001) (figure 15)).

Figure 10: Change in self reported early morning cough



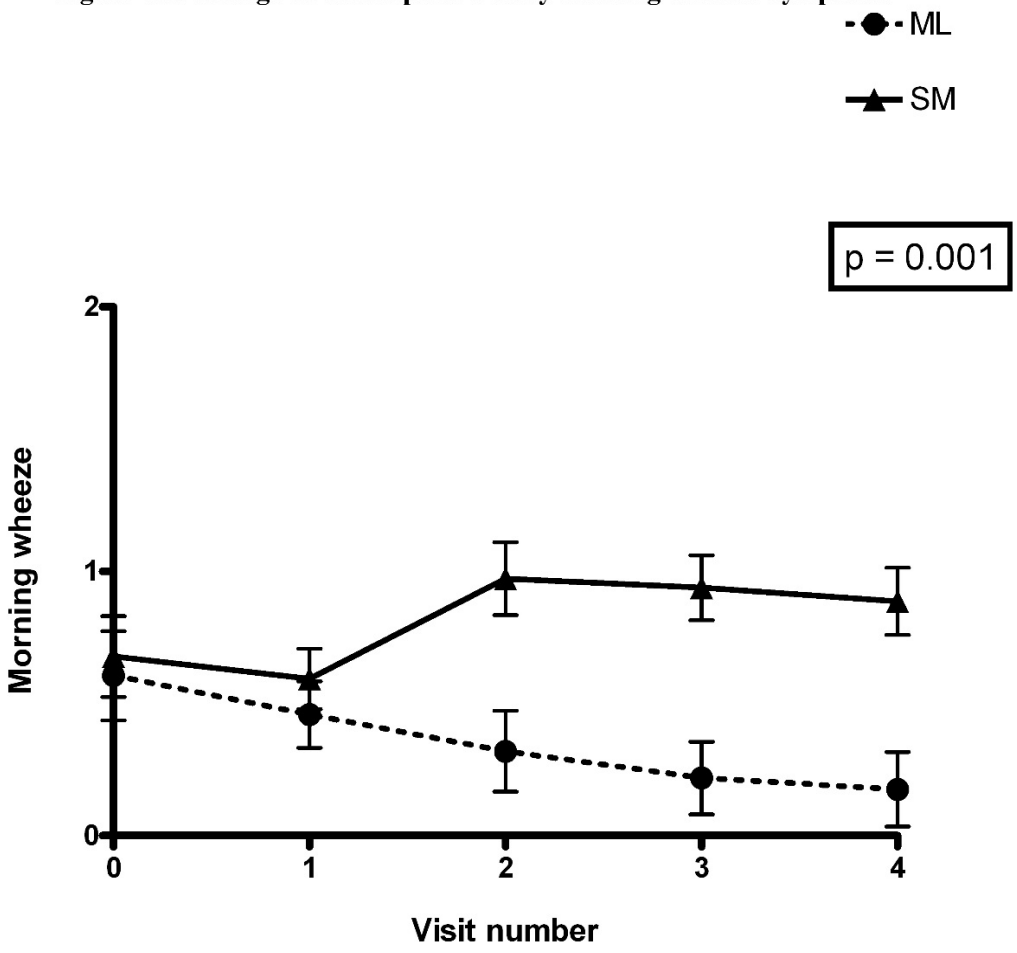
Comparison of mean reported morning cough symptoms between two treatment groups - fluticasone plus montelukast (ML) and and fluticasone with salmeterol and placebo montelukast (SM). Participants and/or their parents reported how much cough in the morning the child experienced at baseline and 3 monthly follow-up visits (0 – 4) during the treatment period of 12 months.

0 = no reported cough,
1 = occasional cough (more than once a week and less than daily use)
2 = daily reported cough in the morning.

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups.

** Significant differences were observed (p = 0.018). Less morning cough was reported in ML group compared to the SM group.*

Figure 11: Change in self reported early morning wheeze symptoms



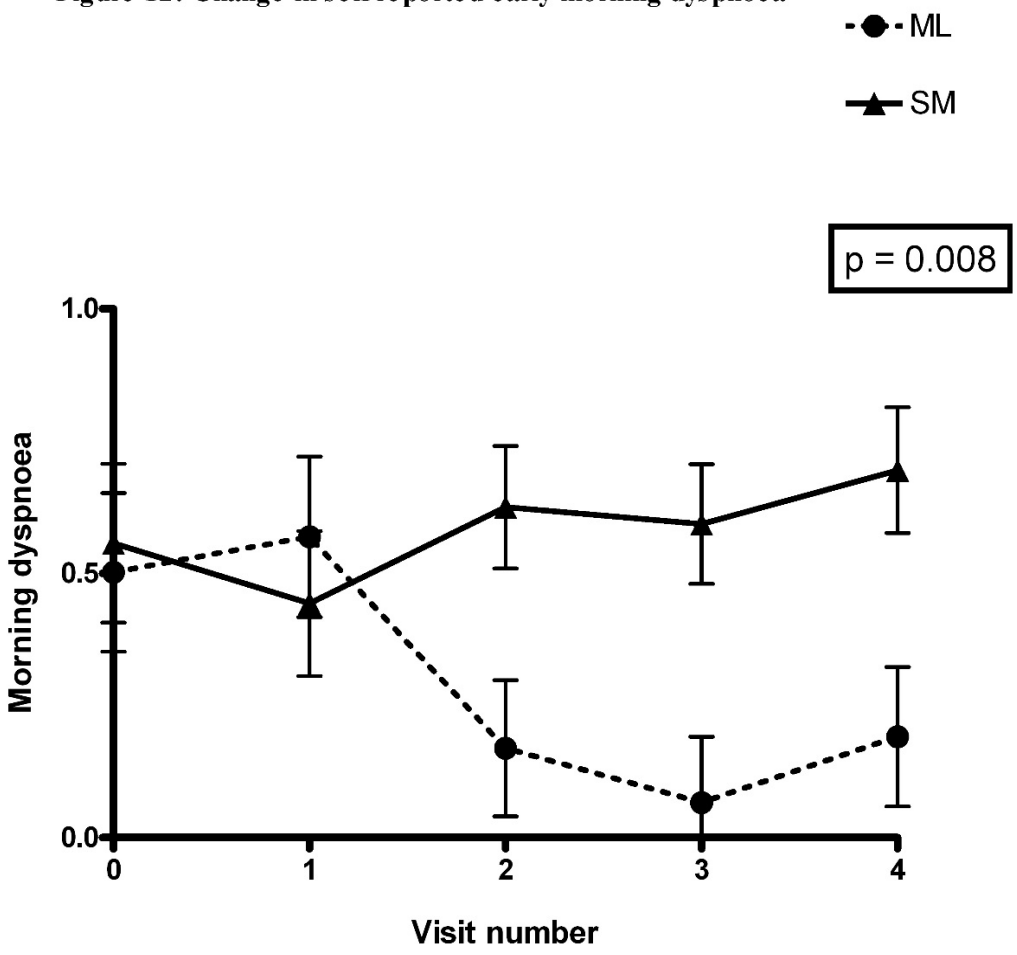
Comparison of mean reported morning wheeze symptoms between two treatment groups - fluticasone plus montelukast (ML), and fluticasone and salmeterol with placebo montelukast (SM). Participants and/or their parents reported how much wheeze in the morning the child experienced at baseline and 3 monthly follow-up visits (0 – 4) during the treatment period of 12 months.

0 = no reported wheeze,
1 = occasional wheeze (more than once a week and less than daily use)
2 = daily reported wheeze in the morning.

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups.

** Significant differences were observed (p = 0.001) i.e. less morning wheeze was reported in ML group compared to the SM group.*

Figure 12: Change in self reported early morning dyspnoea



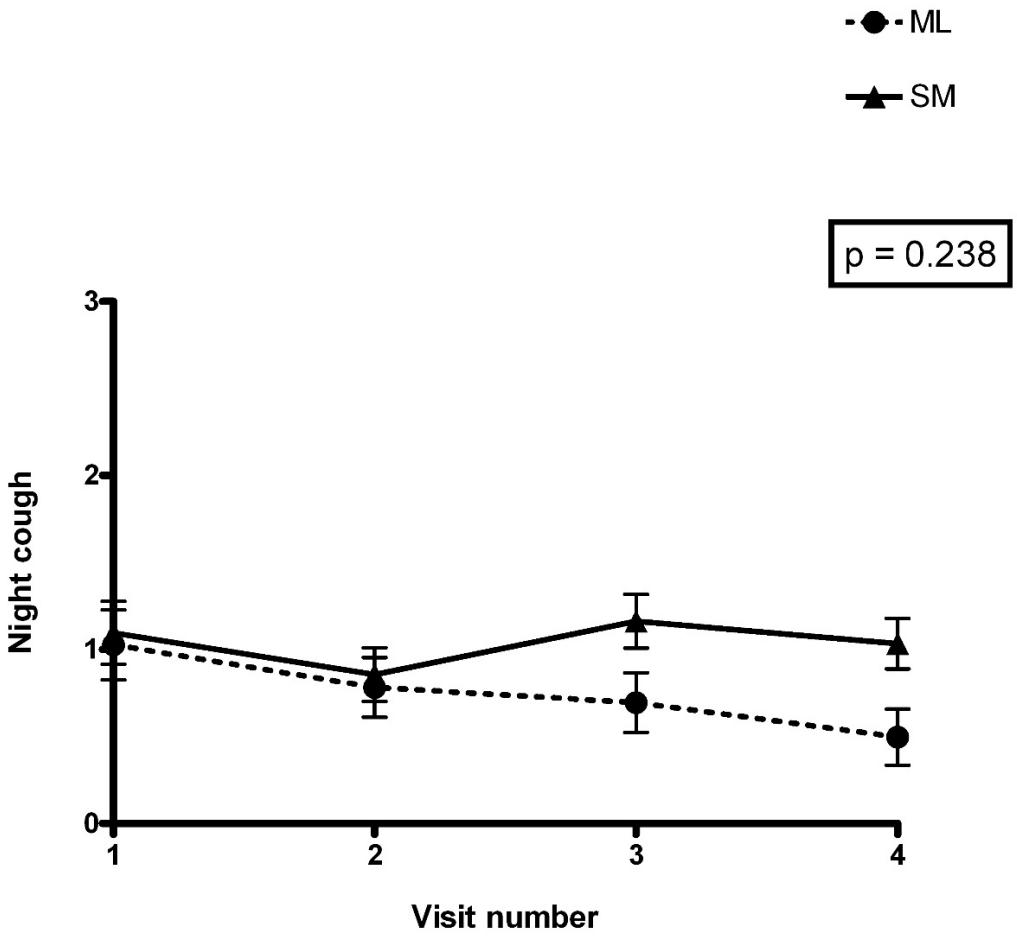
Comparison of mean morning dyspnoea symptoms between two treatment groups - fluticasone plus montelukast (ML), and fluticasone and salmeterol with placebo montelukast (SM). Participants and/or their parents reported how much dyspnoea in the morning the child experienced at baseline and 3 monthly follow-up visits (0 – 4) during the treatment period of 12 months.

0 = no reported dyspnoea,
1 = occasional dyspnoea (more than once a week and less than daily use)
2 = daily reported dyspnoea in the morning

Error bars represent 95% confidence intervals and p value is shown for comparison between the two treatment groups.

** Significant differences were observed (p = 0.008) i.e. participants in ML group reported less morning dyspnoea compared to SM group.*

Figure 13: Change in self reported night cough



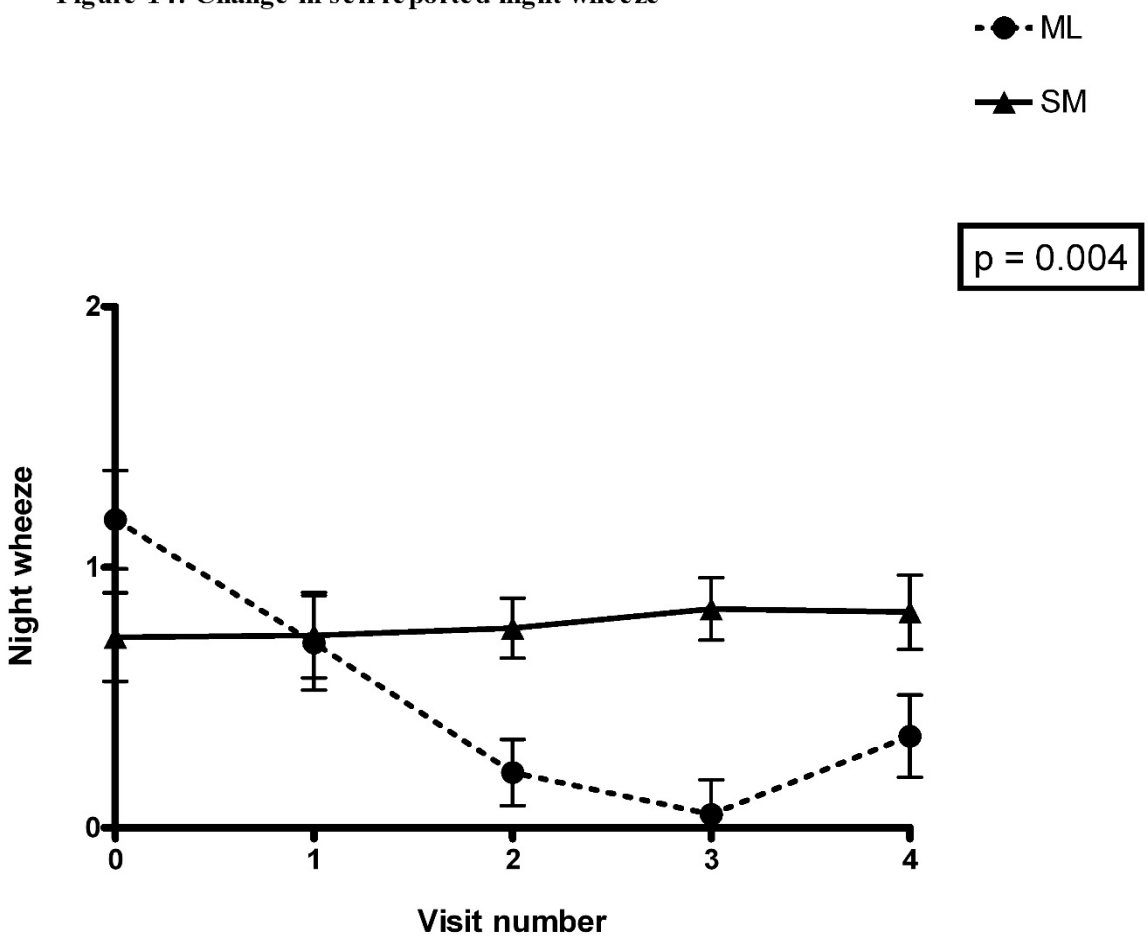
Comparison of mean cough symptoms overnight between two treatment groups - fluticasone plus montelukast (ML), and fluticasone and salmeterol with placebo montelukast (SM). Participants and/or their parents reported how much cough through the night the child experienced at baseline and 3 monthly follow-up visits (0 – 4) during the treatment period of 12 months.

0 = no reported cough
1 = occasional cough (more than once a week and less than daily use)
2 = daily reported cough in the morning

Error bars represent 95% confidence intervals and p values are shown for the comparison between the two treatment groups.

There were no significant differences (p = 0.238) between the two groups.

Figure 14: Change in self reported night wheeze



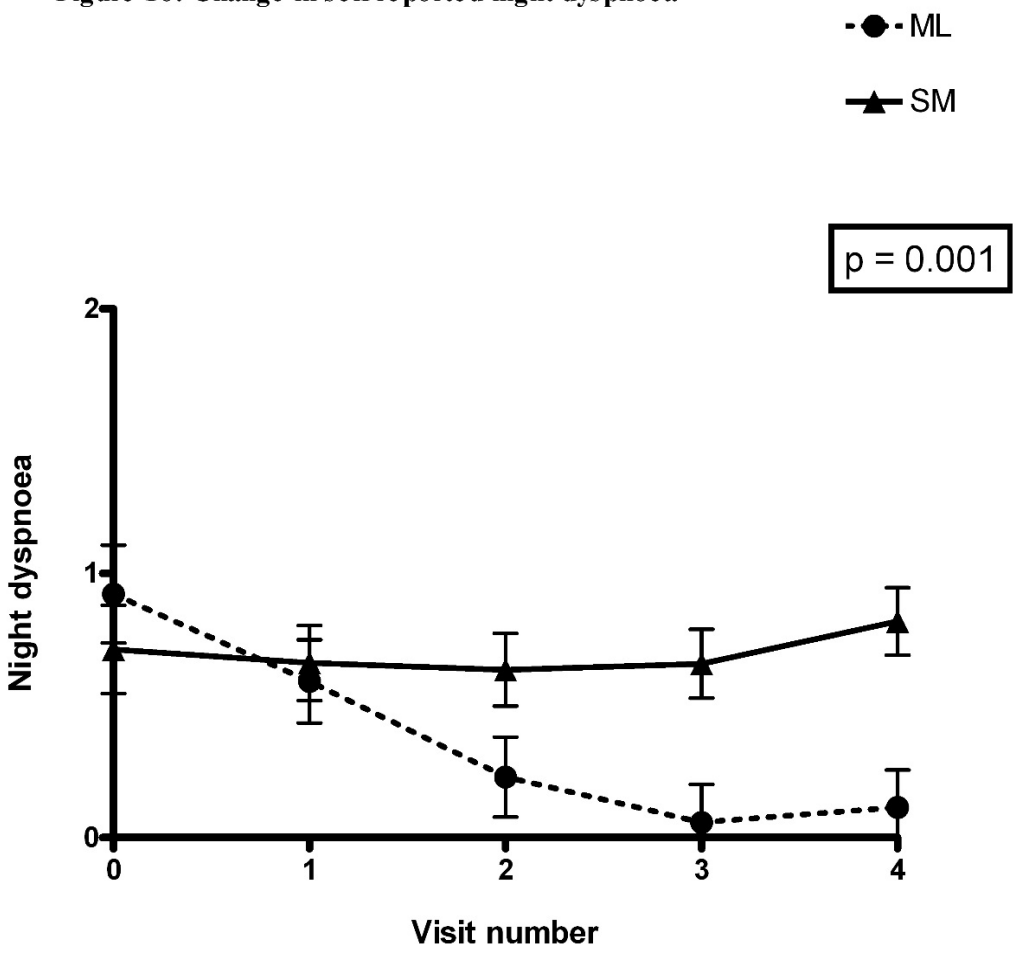
Comparison of mean wheeze symptoms overnight between two treatment groups - fluticasone plus montelukast (ML), and fluticasone and salmeterol with placebo montelukast (SM). Participants and/or their parents reported how much wheeze through the night the child experienced at baseline and 3 monthly follow-up visits (0 – 4) during the treatment period of 12 months.

0 = no reported wheeze
1 = occasional wheeze (more than once a week and less than nightly)
2 = daily reported wheeze overnight.

Error bars represent 95% confidence intervals and p values are shown for the comparison between the two treatment groups.

** Significant differences were observed (p = 0.001) i.e. participants in ML group reported less night wheeze compared to the SM group.*

Figure 16: Change in self reported night dyspnoea



Comparison of mean dyspnoea symptoms overnight between two treatment groups - fluticasone plus montelukast (ML), and fluticasone and salmeterol with placebo montelukast (SM). Participants and/or their parents reported how much dyspnoea through the night the child experienced at baseline and 3 monthly follow-up visits (0 – 4) during the treatment period of 12 months.

0 = no reported dyspnoea
1 = occasional dyspnoea (more than once a week and less than daily use)
2 = daily reported dyspnoea at night.

Error bars represent 95% confidence intervals and p values are shown for the comparison between the two treatment groups.

** Significant differences were observed (p = 0.001) i.e. participants in ML group reported less night dyspnoea compared to the SM group.*

Chapter 14

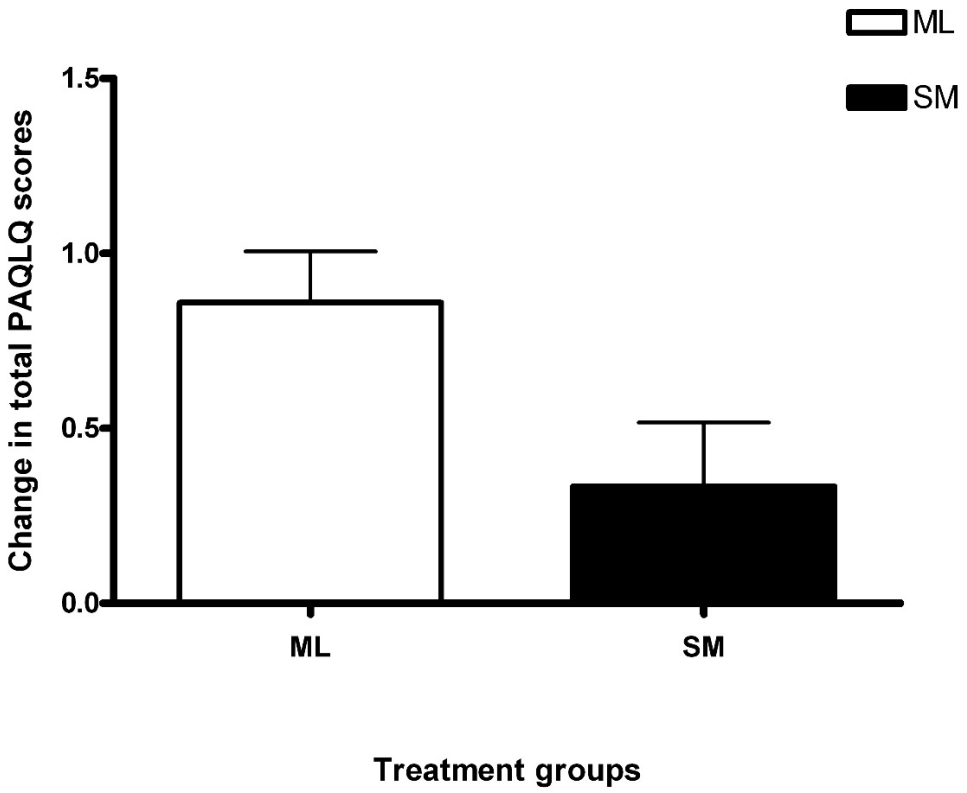
COMPARISON OF END OF STUDY OUTCOMES

Asthma related quality of life

Over the year, significant differences were observed between the asthma quality of life scores in the 2 groups (figure 16) with greater improvement in all the scores in the ML group compared to the SM group. The difference for the overall mean score was 0.53 (95%CI, -0.864- -0.189; p = 0.003) in ML compared to SM. While comparing the mean scores for individual domains between the two treatment groups over the study period, significant differences in improvements were noted in the symptom score (-0.53 (95%CI, -0.92- -0.14; p<0.0001)) (figure 17), emotional function score (-0.523 (95% CI, -0.84- -0.20; p <0.0001)) (figure 18), and activity limitation score (-0.55 (95%CI, -0.918- -0.18; p=0.004)) (figure 19).

No clinically significant changes to vital signs or physical examination parameters were observed following the treatment periods. Reported adverse events such as cough, sore throat were mainly mild in intensity and all considered to be unrelated or of unlikely relationship to the study medication. There were no serious or severe adverse events, nor were there any deaths.

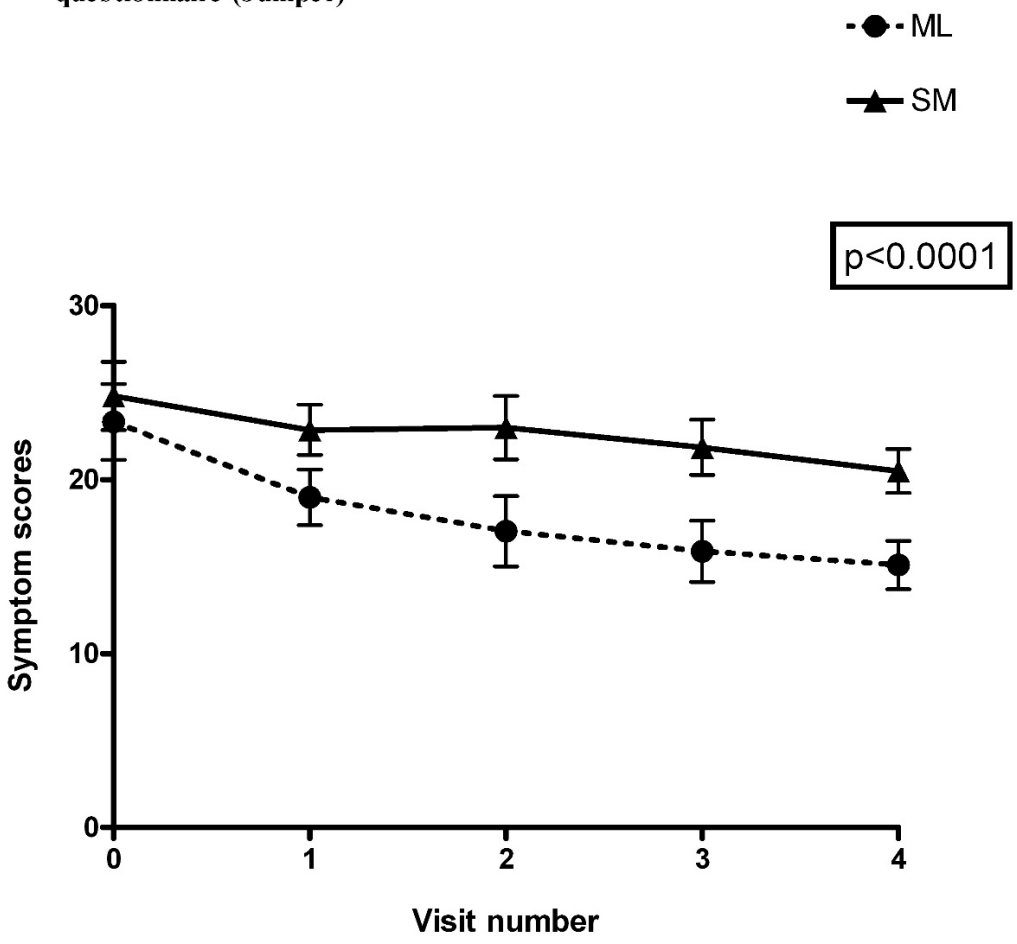
Figure 16: Change in total Paediatric Asthma Quality of Life questionnaire (Juniper) scores



Comparison of the change in the total Paediatric asthma quality of life questionnaire (Juniper) scores between the treatment groups - fluticasone plus montelukast (ML), and fluticasone and salmeterol with placebo montelukast (SM). Results from activity limitation, emotional and symptoms were combined and are shown at baseline and 3 monthly follow-up visits (0 – 4) during treatment period of 12 months.

** Significantly greater improvements were noted for the overall PAQLQ score for the ML group compared to the SM group (p = 0.003)*

Figure 17: Change in symptom scores from Paediatric asthma quality of life questionnaire (Juniper)

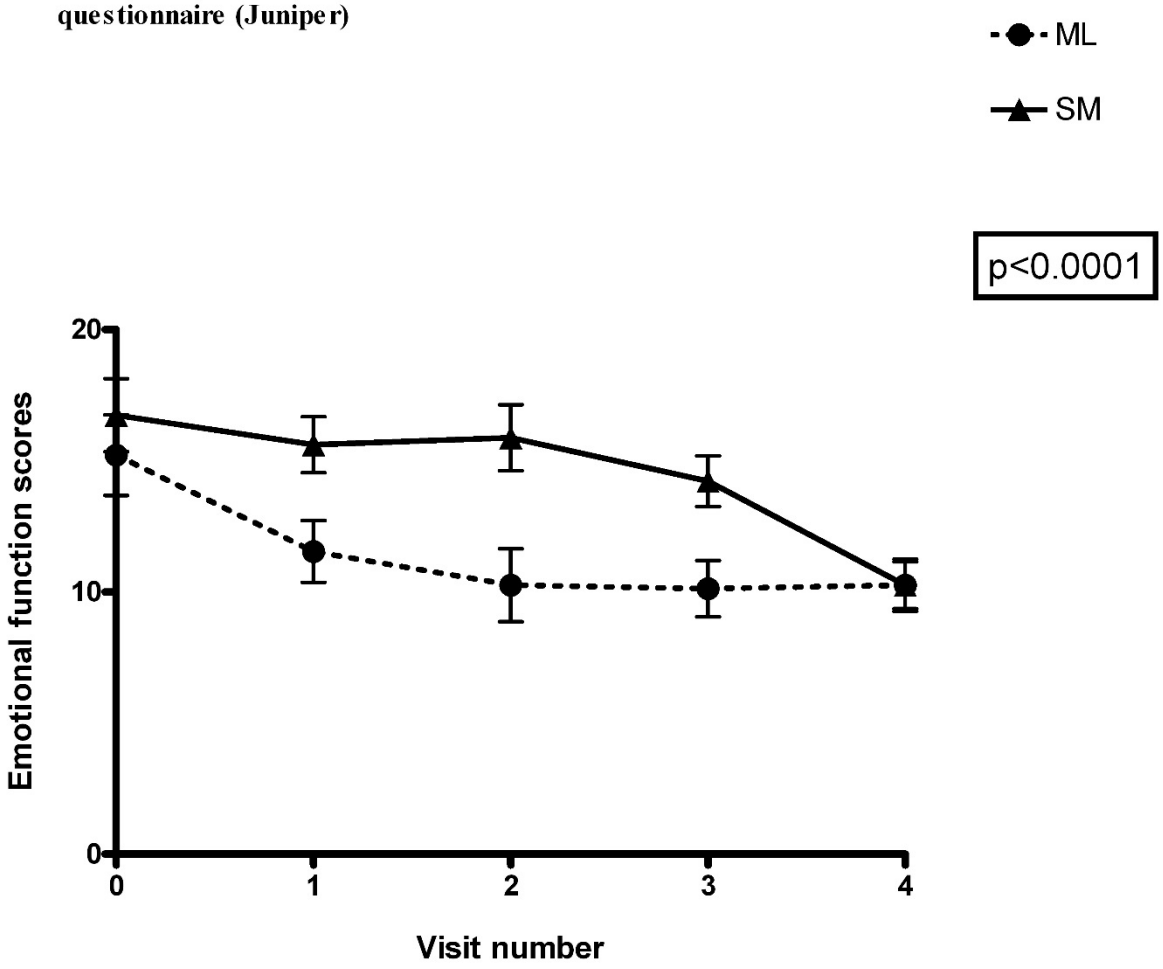


Comparison of mean symptom scores between two treatment groups - fluticasone and montelukast (ML), and fluticasone and salmeterol with placebo montelukast (SM). Paediatric asthma quality of life questionnaire (Juniper) scores for the week preceding each visit were obtained at baseline and 3 monthly follow-up visits (0 – 4) during treatment period of 12 months.

Error bars represent 95% confidence intervals and p values are shown for the comparison between the two treatment groups through the treatment period.

** Significant differences were observed in the asthma symptom outcomes (p = 0.009) i.e. improvements were noted in asthma symptom scores in the ML group compared to SM group.*

Figure 18: Change in emotional function scores from Paediatric asthma quality of life questionnaire (Juniper)

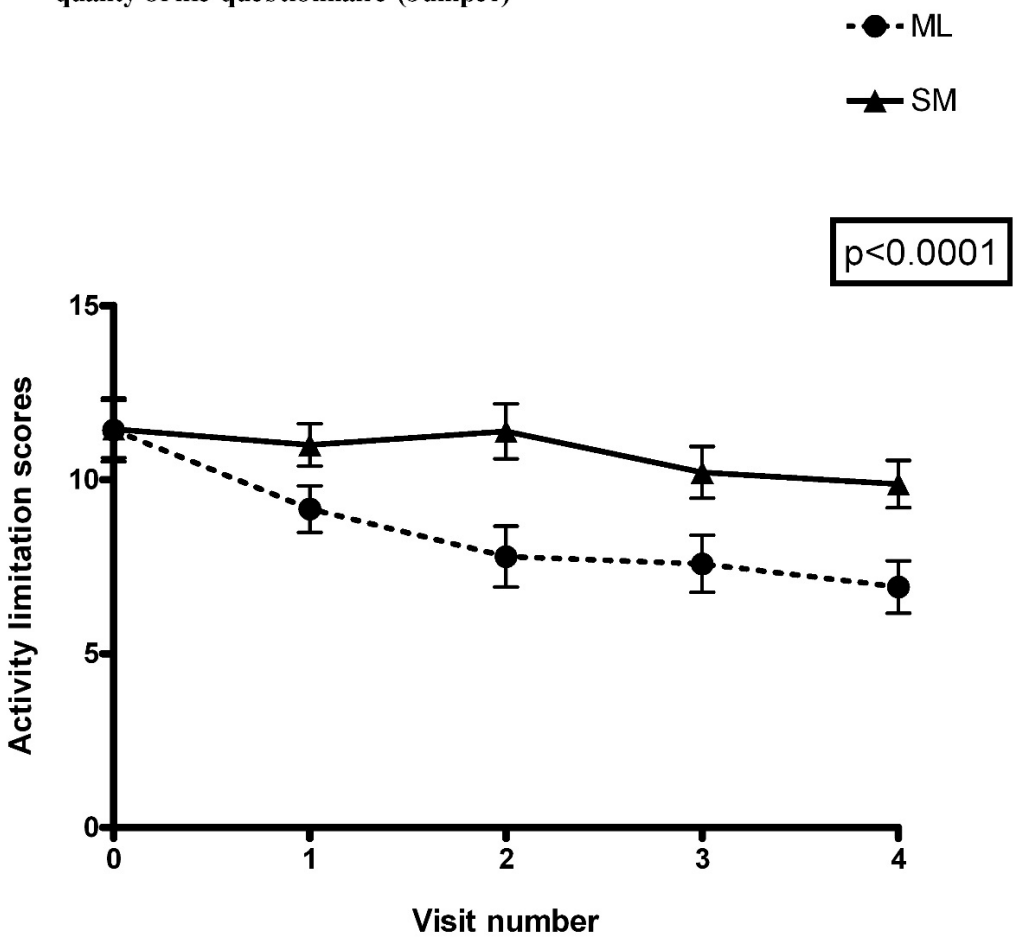


Comparison of mean emotional function score between two treatment groups - fluticasone plus montelukast (ML), and fluticasone and salmeterol with placebo montelukast (SM). Paediatric asthma quality of life questionnaire scores[93] for the week preceding each visit were obtained at baseline and 3 monthly follow-up visits (0 – 4) during treatment period of 12 months.

Error bars represent 95% confidence intervals and p values are shown for the comparison between the two treatment groups through the treatment period.

** Significant differences were observed in emotional function outcomes (p < 0.0001) i.e. improvements were noted in emotional score in the ML group compared to SM group.*

Figure 19: Change in activity limitation scores from Paediatric asthma quality of life questionnaire (Juniper)



Comparison of mean activity limitation scores between two treatment groups - fluticasone plus montelukast (ML), and fluticasone with salmeterol and placebo montelukast (SM). Paediatric asthma quality of life questionnaire scores for the week preceding each visit were obtained at baseline and 3 monthly follow-up visits (0 – 4) during trial period of 12 months.

Error bars represent 95% confidence intervals and p values are shown for the comparison between the two treatment groups through the treatment period.

** Significant differences were observed in activity limitation score ($p < 0.0001$) i.e. improvements were noted in activity limitation scores in the ML group compared to SM group.*

No significant difference was observed in the exhaled nitric oxide between the treatment groups over the study period (data not shown). No clinically significant changes to vital signs or physical examination parameters were observed following the treatment periods. The treatment-emergent adverse events such as cough, sore throat were mainly mild in intensity and all considered to be unrelated or of unlikely relationship to the study medication. There were no serious or severe adverse events, nor were there any deaths during the study period.

SECTION 5 - DISCUSSION

Chapter 15

MAIN FINDINGS

This study was the first prospective randomised control trial in children with asthma using previously obtained genotype information relating to β_2 adrenergic receptor gene. The β_2 adrenergic receptor gene has been shown to have an important role in the mechanism of one of the most commonly used asthma treatments - β_2 agonists.

The study raised a number of ethics-related and nursing issues that merit further analysis and discussion. For example, the process of using genotype for randomisation was intensely discussed by Ethics committees, and the trial raised a number of interesting ethics-related and nursing management-related issues during my interaction with parents, children, general practitioners and nursing colleagues.

Hence, my discussion will follow three specific themes. In the first of my chapters, I will briefly review the pharmacology-related findings and discuss the genotype-related issues underlying the trial findings. In the second chapter, I will reflect on the ethics and nursing-related issues that have emerged from my work. The third chapter will reflect on the problems that I encountered, how these problems might have been managed more effectively, and what I have learnt from the process that will influence my future work.

Chapter 16

PHARMACOGENETIC FINDINGS

Overall summary

The study has shown that asthmatic children with the *Arg/Arg* 16 genotype appear to have better asthma control when prescribed montelukast compared to salmeterol, when added to inhaled corticosteroid, over a 12 month period.[86] This was apparent within the first three months of the study and persisted throughout the year long trial period. This information could support personalised approached medicine prescriptions in the future of children's asthma based on β_2 adrenergic receptor genotype.

Variability in treatment response between individuals can be influenced by many factors, including concordance. Genetics may contribute to as much as 80% of the variation.[85] The findings of this study support a genetic component i.e. β_2AR. The Arg/Arg 16 genotype appears to have an important role in determining how well children with asthma respond to the treatments most widely used at step 3 asthma guidelines.

Knowledge of the β_2AR genotype of a child attending asthma clinics could in the future influence treatment decisions, potentially resulting in quicker control of symptoms and maximising therapeutic benefit as well as reducing possible side effects. This could also result in positive financial savings.

The study was powered showing an n of 30 participants in each arm to provide 80% power to detect a significant difference for school absences between the two groups. Whilst it must be acknowledged that there are limitations in terms of sample size for the study, the findings have implications for future research into the field of pharmacogenetics in asthma.

Comparison with findings from other similar published work

A recent study by Lemanske et al[87] did not find any differences in medication response according to Arg/Gly variation. They considered genetic differences at the end of a trial designed to assess the frequency of differential responses to three blinded step-up treatments in children who had uncontrolled asthma while receiving low dose inhaled corticosteroids. As part of recruitment criteria asthmatic children were required to show reversibility to inhaled beta-agonists, which could be biasing towards participants responding to salmeterol. This has been the case in some adult pharmacogenetic studies, Bleecker et al[72] and the LARGE study[73] also required participants to be responsive to short acting beta agonists to be included in the study.

Choice of end point

School absence is a relevant primary outcome measure for a study involving children. It is easy to measure and relates directly to quality of life in children and is an outcome measure that children and their families can relate to. The choice of endpoint and study design are of potential importance in pharmacogenetics studies e.g. using beta agonist reversibility as entry requirement in an asthma pharmacogenetc study could be seen to bias results in favour of beta agonist responders.

Differences in child and adult physiology

Although the underlying pathophysiology of childhood and adult asthma may be similar, effects on growth and development, and the adverse effects of asthma treatments differ. Differences such as eosinophil response and specific risks associated with maternal smoking[88] suggest that the clinical effects of the *Arg/Gly* variation could differ between asthmatic children and adults. Indeed, the effect of the *Arg/Gly* polymorphic variation on inhaled β_2-agonist response seems more consistent in children in comparison to adults with asthma.[27,63] Differences such as this highlight why it is not acceptable to transfer results from studies of asthma in adults to paediatric populations

Chapter 17

ETHICAL ISSUES AND NURSING RELATED ISSUES

Ethical issues from a nursing perspective

This chapter presents below a critical analysis of the methodology and design underlying the genotype-stratified randomised controlled trial, from a nursing perspective. The nursing experience around the process of recruitment for this study forms the basis for my key observations around the ethical issues and nursing issues encountered during this study. I have primarily focussed on my interaction with children and their families.

Clinical Trials involving children

The ethics of research with children should be based on careful assessment of potential benefits and risks.[89] Research nurses play a key role in interpreting guidelines from regulatory authorities and child health organisations produced to safeguard the interests of children participating in research.[90,91] US and EU regulations on paediatric medicines positively support that children should be able to benefit from research in clinical settings.[93,94] Thus to translate advances in pharmacogenetics to benefit children, it is necessary to perform studies with child participants that involve genetic screening.

Risk Assessment for child and young adult participants

The participants were carefully monitored by a specialised paediatric asthma nurse and doctor, and the study did not involve any unpleasant tests, therefore presented no major increase in risk to the child participants.[93] Indeed, the BREATHE observational study[63]

identified this patient sub-group at potential overall risk; however, benefits from montelukast or salmeterol could vary in individual cases and genotype-based prescribing may not necessarily be of therapeutic advantage. The study therefore was a natural progression of clinical planning in terms of nursing practice, keeping in mind the best interests of the patient

Prospective Genotyping for child participants

The study involving both child and adolescent participants and the inclusion of a form of genetic testing raised a number of ethical issues that were addressed over the course of this study. While there is considerable literature related to the genetic testing of children, both for childhood and later onset adult conditions, there has been very little discussion of the ethics of pharmacogenetic research on children.[94] The work that has been done in this area highlights the complex issues around informed consent to such trials (from both children and their parents), the limitations on risks that can be taken with children, and the complicated genetics involved in cases where genes may be expressed differently in children compared to adults.[95]

Time was taken with discussion of the *Arg16* genotype with parents and children prior to recruitment. It was explained that the ‘mouthwash study' [63] had identified a specific gene about which we were trying to find out more and that when we had looked back we found that the children who had a particular genotype had more symptoms. The current proposed study was trying to find out more about how the child carrying the risk

genotype reacted to the medicines in question, and whether salmeterol ("asthma inhaler") or montelukast ("asthma tablet") was a better option for treatment in these children.

Clarification of Gene's Role

It was important to consider whether parents/children would have prior perceptions about genetic testing. They may have had concerns about the gene being tested for, its role in the pharmacogenetics of beta agonists, and even if this would have a long term effect on their childs condition.[96] In this study, pharmacogenetic testing for the *Arg/Arg 16* did not raise this ethical issue because the gene appears to relate only to treatment response.[97] One parent did express concerns of a legal nature relating to perceived disclosure obligations of genetic testing results to insurers for their child in the future and requested to have the child's information withdrawn from the original pilot database.

Informed consent to genetic testing

The issue of obtaining informed consent was an ethical issue to consider. While there have been very few studies on participant's attitude towards and understanding of informed consent forms in pharmacogenetic trials, what work has been done, and broader work on the problems of informed consent should raise questions about how well participants understand what it is they are consenting to, when they allow their DNA to be sampled.[97,98] Whilst I was not involved in initial BREATHE study to obtain a saliva sample, it was important that I had an understanding of questions that may arise when families where contacted to participate in a second study. Overall, participants and their

parents were interested in contributing to the understanding of the gene-environment interactions in childhood asthma and allergy.

Informed consent to enter the study

Parents were motivated to consent to their children entering the study because there was a possible perceived benefit for their own child found to carry the genotype, but also the satisfaction of knowing they may be helping other children with asthma in the future. Consent was also obtained from the children. A paediatric nurse or a doctor performed the consenting and discussed the trial with the carers and the children. It was important to take time to always include the children in the consent process. Prior to beginning recruitment, the research team worked closely with the Ethics Committee to develop child friendly study information leaflets and a consent form where both the children and parents were involved in the consent process. The study medicines – inhaled salmeterol and oral montelukast - are already widely prescribed in children and young adults with asthma, so it was reassuring for parents and children that they were not being asked to participate in a study of a novel asthma treatment. It was important to explain fully to the parents that our objective was to test the hypothesis that children and young adults with asthma who have the *Arg/Arg*-16 polymorphic variation will be better controlled by the use of montelukast instead of long acting beta$_2$ agonists as controller medication, but that this was still an open question.

Randomisation procedure

The parents and young participants also needed to be aware that allocation to a treatment group was determined by a randomisation procedure. From an understanding of the hypothesis they may have naturally wanted their child to be in the montelukast treatment group. In one particular instance, the mother said at the time of randomisation that the asthma symptoms of her child, who was found to be carrying the *Arg/Arg* homozygous genotype, had previously responded well to montelukast. This child was randomised to the active salmeterol /placebo montelukast arm. He experienced increased symptoms and exacerbations during the initial study period, and eventually decided to withdraw after 6 months. This was the focus of further discussion regarding ethical and other issues surrounding randomisation on the basis of genotype where the available genotypic evidence together with the report of clinical response at the start of randomisation could have suggested the outcome.

Chapter 18

PRACTICAL PROBLEMS AND THEIR MANAGEMENT

The study was an important learning experience and it will influence my views regarding the design of randomised controlled trials and other studies in the future. I will summarise specific issues of interest and describe how these issues presented in the first instance and how they were managed, and difficulties I encountered in the process.

- Difficulties with recruitment
- Feedback and response from parents and children
- Feedback and response from general practitioners and hospital colleagues

Difficulties in Recruitment of appropriate number of participants

Each child/family identified as carrying the *Arg/Arg* 16 genotype was systematically approached by sending information by post and contacting the families a short time later to offer them an appointment to discuss the information further. Ethics committees stipulate that in a trail situation families are given opportunity to read information regarding the study so that they are as informed as possible prior to being contacted to ask if they would like to consider taking part in the proposed research.

A period of one year had been agreed with the ethics committee during which participants could be recruited. The majority of participants were recruited within the first 3 months of the trial. However after a period we had contacted all potential recruits

from the original *Arg/Arg* genotype list and had not reached the predetermined target number - 60. To be able to detect this difference in effect with a probability (power) 30 subjects in each group was target number of participants. However, if each group had 40-50 participants, the null hypothesis could be disproved with confidence

Actions to increase potential recruitment

Contact some families previously ineligible

We decided to contact some of the families who had previously expressed interest in participating, for a second time. These potential participants may not have met entry criteria e.g. had experienced no exacerbations the previous year or were no longer prescribed inhaled steroids. It may have been that over the winter months they had experienced some asthma symptoms which would then make them eligible to participate. Only one further participant kindly agreed to join the study after being contacted a second time.

Further laboratory analysis

At laboratory level Scientists rechecked samples and analysed more recent mouthwash samples in order to identify more children with *Arg Arg* 16 genotype not on the original list who could be approached to participate.

Consider second recruitment centre – Dumfries

By May 2008 – 9 months into the study all children identified with *Arg/Arg* 16 genotype (148 children) had been approached and 49 participants had been recruited. With no further potential participants in Tayside, the research team began to consider the possibility of extending recruitment area to a second Scottish centre - Dumfries. Children carrying *Arg/Arg* 16 genotype had already been identified in Dumfries and Galloway from the same genetic study used in Tayside.

We contacted our Ethics Committee to discuss the feasibility of adding a second centre to the study. However it quickly became evident that this would involve a long process of reapplying to Ethics committee in Dumfries, NHS Research and Development departments and MHRA once again. Effectively restarting the pre-trial process again would prolong the study considerably. It is advisable to apply at the initial stages of pre-trial procedures if the study is a multi-centre one. The possible need to use another area in Scotland had not been anticipated during our pre-trial work.

We were fortunate to finally recruit 62 children and young adults which was within our pre-determined sample size. The final participants were recruited from the further saliva sample analysis results.

Work with General Practise Colleagues

It was extremely important to have the support of our colleagues in General Practise to carry out the study. An information leaflet clearly explaining what the study involved

was sent to each child's General Practitioner (GP). A request was also issued to the GP to ask them prescribe the accuhaler device with either seretide or fluticasone. Montelukast was supplied from our clinical trials pharmacy. Financial constraints and operational difficulties meant, in contrast to montelukast where patients were given montelukast or placebo, we were unable to provide blinded accuhaler devices. We were therefore reliant on GP's to prescribe the accuhaler device. This request could potentially have occurred as a result of a visit to a routine asthma clinic review. One GP was initially reluctant to prescribe the different treatment but he did agree after a meeting with the young participant to establish her willingness to participate and some further discussion with the research team regarding the study.

Failure to register with Clinical Trials

ClinicalTrials.gov is a database register of clinical trial details and results of clinical studies of human participants conducted around the world. It is a web-based resource where patients, family members, health care professionals, researchers, and the public can access information on publicly and privately supported clinical studies on a wide range of diseases and conditions. The Web site is maintained by the National Library of Medicine (NLM) at the National Institutes of Health (NIH).https://clinicaltrials.gov**.** Unfortunately members of the trial research team were unaware of this site and midway through the trial we received communication that it was obligatory to register the trial with this site.

The procedure for pre-trial clinical trials has changed considerably since application process for this trial. There is now an Integrated Research Application System and a flow

chart to guide researchers through the process. The Ethics committee now appoint a representative from their department to work closely with research teams throughout the whole process – pre-trial procedures, trial period and completion of trials. Therefore failing to register with the Clinical trial website should not occur in the future.

Critique of the study

The following is a short critique of the study design and points that have been raised that could be useful in future work.

No control group

One of the criticisms that could be made about the study design is that there was no control group included. This was a pilot study where the primary hypothesis was that children with asthma carrying the *Arg/Arg*-16 genotype have fewer school absences over a period of 1 year on oral montelukast in comparison to inhaled salmeterol. Our work focused on the primary question and for this there was no requirement for having a control group.

Blinding

Children could easily determine which treatment group they had been randomised into once they received their accuhaler inhaler device. As much as could be determined from dose counter readings on the device and the numbers of montelukast/placebo tablets returned, it did not appear that the knowledge of treatments differed between each treatment group.

Chapter 19

CONCLUSION

I was very fortunate to be involved in one of the first prospective randomised controlled studies involving children with asthma that addresses the hypothesis that asthma treatments in the future could be tailored on an individual basis with information about genotype.

Concluding remarks on discussion

I have presented the results of a prospective randomised controlled trial comparing second line treatment options in children with asthma who carry the Arg16/Arg16 $beta_2$ adrenoreceptor genotype. Although with considerable variability in findings between studies, the presence of this genotype has previously been shown to potentially modulate short acting beta agonist and/or long acting beta agonist responsiveness. The results of the study support the need for a larger blinded study with an appropriate control group.

I have also tried to explore the ethical and recruitment-related issues around the design of genotype-stratified randomised controlled trials in children and have discussed some of the practical aspects of the ethics-related issues associated with the study, the response of parents and children to such issues, and how these issues were managed within the context of this study. I hope this provides a platform for further discussion on the methodology of prospective genotype-stratified trials in children, particularly from the

nursing perspective, as such trials, with varying levels of nurse-researcher involvement, are likely to become more relevant to the progress in therapeutics in the future.

Nurse researchers are increasingly involved in the design and conduct of successful paediatric clinical trials and are often directly involved in clinical trials recruiting children with asthma that aim to answer specific questions of clinical importance. If treatments can be designed to meet the specific needs of children with *Arg-16* polymorphic variation, studies such as this require to be performed specifically in children. Such work could lead to increased efficacy and response to treatment, and the avoidance of adverse effects in childhood asthma therapy.

Why was this research needed?

- Research papers using observational design, studying children with asthma, suggest that response to asthma medication in children may be influenced by genotype.
- There is limited research on the ethical issues around genotyping for randomised controlled trials and other research studies particularly in children.

What are the key findings

- The study explores the practical aspects of ethics-related issues associated with such genotyping, within the context of randomisation for studies in childhood asthma.

- The work considers the response of parents and children in relation to these issues, and tries to provide solutions for these issues within the context of this research work.

How should the findings be used to influence policy, practice, research and education

- This could contribute to clinical practice, as genetic testing may improve treatment efficacy and minimise risk to children with asthma using β_2-agonist relievers and other medication.
- The study should provide guidance for the design of similar trials, for example, through the presentation of relevant clinical scenarios that may be encountered by the researchers.
- I have presented the pharmacological findings from the study that are of great interest in the treatment of asthma in children, and will be valuable as the field of pharmacogenetics in children progresses.

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